

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			0147-0223P
INTERNATIONAL APPLICATION NO. PCT/EP99/07633			U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <b>09/806767</b>
INTERNATIONAL FILING DATE October 12, 1999		PRIORITY DATE CLAIMED October 12, 1998	
TITLE OF INVENTION MEANS AND METHODS FOR MODULATING STOMATA CHARACTERISTICA IN PLANTS			
APPLICANT(S) FOR DO/EO/US BERGER, Dieter; ALTMANN, Thomas			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).</li> <li>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. WO 00/22144</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4)</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>			
Items 11. to 20. below concern document(s) or information included:			
<ol style="list-style-type: none"> <li>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98-International Search Report (PCT/ISA/210) w/ 6 documents</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>15. <input type="checkbox"/> A substitute specification.</li> <li>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.</li> <li>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>20. <input checked="" type="checkbox"/> Other items or information: <ol style="list-style-type: none"> <li>1.) PCT Substitute Claims Letter w/ International Preliminary Examination Report (PCT/IPEA/409) and claims</li> <li>2.) PCT Request (PCT/RO/101)</li> <li>3.) 43 pages of Sequence Listing</li> <li>4.) Nine (9) sheets of Formal Drawings</li> </ol> </li> </ol>			

U.S. APPLICATION NO (if known, see 37 CFR 1.5) <b>09/7806767</b>		INTERNATIONAL APPLICATION NO PCT/EP99/07633		ATTORNEY'S DOCKET NUMBER 0147-0223P																					
21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. .... <b>\$1,000.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. .... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS      PTO USE ONLY</b>																					
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	860.00																				
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:15%;">CLAIMS</th> <th style="width:20%;">NUMBER FILED</th> <th style="width:20%;">NUMBER EXTRA</th> <th style="width:20%;">RATE</th> </tr> </thead> <tbody> <tr> <td>Total Claims</td> <td>29 - 20 =</td> <td>9</td> <td>X \$18.00</td> </tr> <tr> <td>Independent Claims</td> <td>3 - 3 =</td> <td>0</td> <td>X \$80.00</td> </tr> <tr> <td colspan="4">           MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes + \$270.00         </td> </tr> <tr> <td colspan="4" style="text-align: right;"><b>TOTAL OF ABOVE CALCULATIONS =</b></td> </tr> </tbody> </table>				CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	Total Claims	29 - 20 =	9	X \$18.00	Independent Claims	3 - 3 =	0	X \$80.00	MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes + \$270.00				<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	130.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE																						
Total Claims	29 - 20 =	9	X \$18.00																						
Independent Claims	3 - 3 =	0	X \$80.00																						
MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes + \$270.00																									
<b>TOTAL OF ABOVE CALCULATIONS =</b>																									
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	0																				
<b>SUBTOTAL =</b>				\$	1422.00																				
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	0																				
<b>TOTAL NATIONAL FEE =</b>				\$	1422.00																				
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	0																				
<b>TOTAL FEES ENCLOSED =</b>				\$	1422.00																				
				Amount to be:	\$																				
				refunded	\$																				
				charged	\$																				

- a. ☒ A check in the amount of \$ **1422.00** to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account. No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:

**Birch, Stewart, Kolasch & Birch, LLP** or Customer No. 2292  
 P.O. Box 747  
 Falls Church, VA 22040-0747  
 (703)205-8000

Date: April 3, 2001

By m h/ Niell #30,623  
 a Leonard R. Svensson, #30,330

PROCT 823 10 AUG 2001

#3



BOX SEQUENCE  
PATENT  
0147-0223P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: BERGER, Dieter et al. Conf.: 4759  
Appl. No.: 09/806,767 Group: Unassigned  
Filed: April 3, 2001 Examiner: Unassigned  
For: MEANS AND METHODS FOR MODULATING  
STOMATA CHARACTERISTICA IN PLANTS

AMENDMENT

Assistant Commissioner for Patents  
Washington, DC 20231

August 10, 2001

Sir:

In response to the U.S. Patent Office Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Disclosures dated May 10, 2001, the period for response having been extended one (1) month to August 10, 2001, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please replace the description of figure 5, on page 33, with the following amended description:

--**Figure 5:** Sequence alignments in the four highly conserved domains, the D region(SEQ ID NOS: 62-70), the H region(SEQ ID NOS:71-79), the substrate binding site(SEQ ID NO:80-88), and the S region(SEQ ID NOS:89-97) of the subtilisins Subtilisin BPN' (Wells et al. 1983, Nucleic Acids Res. 11, 7911-7925),

the KEX2 of yeast (Mizuno et al. 1988, *Biochem. Biophys. Res. Commun.* 156, 246-254), the human FURIN/PACE (Wise et al. 1990, *Proc. Natl. Acad. Sci USA* 87, 9378 - 9382), the human PC1/PC3 (Seidah et al., 1991, *Mol. Endocrinol.* 5, 111 - 122; Smeekens et al., 1991, *Proc. Natl. Acad. Sci. USA* 88, 340 - 344), the CUCUMISIN from *Cucumis melo* (Yamagata et al., 1994, *J. Biol. Chem.* 269, 32725 - 32731), LeP69 from *Lycopersicon esculentum* (Tornero et al., 1996, *Proc. Natl. Acad. Sci. USA* 93, 6332 - 6337), the AG12 from *Alnus glutinosa* (Ribeiro et al., 1995, *Plant Cell* 7, 785 - 794) and of SDD1. The positions of the invariant amino acids are marked with \*. Identical amino acids present at corresponding positions in the different subtilisins are highlighted with black boxes.--

Please replace the Sequence Listing filed April 3, 2001, located immediately after the abstract, with substitute Sequence Listing enclosed herewith.

#### REMARKS

##### Sequence Listing

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a substitute Sequence Listing to be inserted into the specification as indicated above. The substitute Sequence Listing in no way introduces new matter into the specification.

Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the substitute Sequence Listing. The disk copy of the substitute Sequence Listing, file "0147-0223P.ST25", is identical to the paper copy, except that it lacks formatting.

The substitute Sequence Listing contains sequences disclosed in figure 5 as filed that were not made part of the original Sequence Listing. The amendments to the Specification are being



made to reference these sequences by their SEQ ID NOS. These amendments are editorial in nature and do not constitute new matter.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Claims Fee

Claims 8, 11, and 15 as filed are improper, therefore these claims and their dependants are counted as one claim making the total claim count to 27. 29 claims were paid for on April 3, 2001, therefore the Applicants feel that the additional claims fee of \$288.00 is not necessary. If the Commissioner disagrees then he is hereby authorized to charge Deposit Account No. 02-2448 for any additional claims fees.

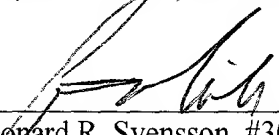
Extension of Time

A one (1) month(s) extension of time for the filing of the present paper in accordance with the provisions of 37 C.F.R. §1.136 and 37 C.F.R. §1.17 has been petitioned for and the applicable fees have been paid in the accompanying response to the Notice to File Missing Parts filed on August 10, 2001 for the present application number.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  #32,868  
J- Leonard R. Svensson, #30,330

LRS/SWG/KW  
0147-0223P

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

Attachments: Paper and disk copy of Sequence Listing  
Copy of Notice to Comply

09/806767

0147-0223P

JCO8 Rec'd PCT/PTO

03 APR 2001

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: BERGER, Dieter et al. Conf.:  
Int'l. Appl. No.: PCT/EP99/07633  
Appl. No.: New Group:  
Filed: April 3, 2001 Examiner:  
For: MEANS AND METHODS FOR MODULATING STOMATA  
CHARACTERISTICA IN PLANTS

PRELIMINARY AMENDMENT

**BOX PATENT APPLICATION**

Assistant Commissioner for Patents  
Washington, DC 20231

April 3, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

**AMENDMENTS**

**IN THE SPECIFICATION:**

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/EP99/07633 which has an International filing date of October 12, 1999, which designated the United States of America.--

**IN THE CLAIMS:**

Please amend the claims as follows:

5. (Amended) A vector comprising a recombinant DNA molecule of claim 1.

6. (Amended) A host cell containing a vector of claim 5 or a recombinant DNA molecule of claim 1.

7. (Amended) A method for the production of transgenic plants with altered stomata characteristics compared to wild type plants comprising the introduction of a recombinant DNA molecule of claim 1 or the vector of claim 5.

8. (Amended) A transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of claim 1 or a vector of claim 5 or obtainable according to the method of claim 7, wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.

11. (Amended) A transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of claim 1 or part thereof, a vector of claim 5 or obtainable according to the method of claim 7, wherein the presence, transcription and/or

expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.

13. (Amended) A transgenic plant or plant tissue comprising the plants cells of claim 11.

15. (Amended) The transgenic plant of claim 9, the plant cell of claim 8, or the plant tissue of claim 9, wherein said plant, plant cell or plant tissue is derived from a monocotyledonous or dicotyledonous plant.

17. (Amended) Harvestable parts or propagation material of plants of claim 9.

18. (Amended) A kit comprising recombinant DNA molecule of claim 1 or a vector of claim 5.

21. (Amended) Use of a nucleic acid molecule encoding or regulating the expression of a subtilisin-like serine protease or a nucleic acid molecule as defined in claim 1, a recombinant DNA molecule of claim 1, or a vector of claim 5 for the production of plants with improved fresh and dry weight, for enhancing the content of sugars and/or protein in plant leaves for the production of plants with reduced leaf temperatures or with reduced water loss and lower water consumption, for the

modification (enhancement) of CO<sub>2</sub> uptake into and H<sub>2</sub>O release from leaves, for sustained photosynthesis under high intensity conditions or for the improvement of disease resistance plants.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete improper multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By m. J. Nell #36,623  
A Leonard R. Svensson, #30,330

LRS/cgc  
0147-0223P

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

Attachment: Version With Markings Showing Changes Made

**VERSION WITH MARKINGS SHOWING CHANGES MADE**

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

5. (Amended) A vector comprising a recombinant DNA molecule of [any one of claims 1 to 4]claim 1.

6. (Amended) A host cell containing a vector of claim 5 or a recombinant DNA molecule of [any one of claims 1 to 4]claim 1.

7. (Amended) A method for the production of transgenic plants with altered stomata characteristics compared to wild type plants comprising the introduction of a recombinant DNA molecule of [any one of claims 1 to 4]claim 1 or the vector of claim 5.

8. (Amended) A transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of [any one of claims 1 to 4]claim 1 or a vector of claim 5 or obtainable according to the method of claim 7, wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.



11. (Amended) A transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of [any one of claims 1 to 4]claim 1 or part thereof, a vector of claim 5 or obtainable according to the method of claim 7, wherein the presence, transcription and/or expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.

13. (Amended) A transgenic plant or plant tissue comprising the plants cells of claim 11 [or 12].

15. (Amended) The transgenic plant of [any one of claims 9, 10, 13 or 14]claim 9, the plant cell of [any one of claims 8, 11 or 12]claim 8, or the plant tissue of claim 9 [or 13], wherein said plant, plant cell or plant tissue is derived from a monocotyledonous or dicotyledonous plant.

17. (Amended) Harvestable parts or propagation material of plants of [any one of claims 9, 10, 13 or 14 to 16]claim 9 [comprising plant cells of claim 8 11, 12, 15 or 16].

18. (Amended) A kit comprising recombinant DNA molecule of [any one of claims 1 to 4]claim 1 or a vector of claim 5.

21. (Amended) Use of a nucleic acid molecule encoding or regulating the expression of a subtilisin-like serine protease or a nucleic acid molecule as defined in [any one of claims 1 to 4]claim 1, a recombinant DNA molecule of [any one of claims 1 to 4]claim 1, or a vector of claim 5 for the production of plants with improved fresh and dry weight, for enhancing the content of sugars and/or protein in plant leaves for the production of plants with reduced leaf temperatures or with reduced water loss and lower water consumption, for the modification (enhancement) of CO<sub>2</sub> uptake into and H<sub>2</sub>O release from leaves, for sustained photosynthesis under high intensity conditions or for the improvement of disease resistance plants.

## Means and methods for modulating stomata characteristic in plants

The present invention relates to recombinant DNA molecules comprising nucleic acid molecules encoding subtilisin-like serine proteases that are involved in the regulation of stomatal density in plants; wherein said nucleic acid molecules could be operably linked to regulatory elements allowing the expression of the nucleic acid molecules in plants. The present invention also provides vectors comprising said recombinant DNA molecules as well as plant cells, plant tissues and plants transformed therewith. The present invention further relates to the use of the aforementioned recombinant DNA molecules and vectors in plant cell and tissue culture, plant breeding and/or agriculture, in particular for the production of plants with improved traits.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated herein by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

Stomatal complexes (stomata) are specialised structures in the epidermices of all higher land plants that mediate and control the gas exchange between the internal tissues of the plants and the atmosphere. They consist of two guard cells that flank a central pore. In many plant species these central guard cells are surrounded by morphologically distinct epidermal cells (subsidiary cells). Usually, more than 90% of the gas exchange between a leaf and the atmosphere (uptake of CO<sub>2</sub> into the leaf and release of H<sub>2</sub>O vapor) occurs through the stomatal pores. The major function of the stomata is to create an optimal balance between sufficient CO<sub>2</sub> uptake and limited water loss. To achieve this, short term control (in the range of minutes to hours) is exerted on the stomatal conductance by opening and closure of the stomatal pores through turgor driven movement of the guard cells (for review see Zeiger, Farquhar and Cowan (eds.) Stomatal Function,

Stanford University Press, Stanford 1987; Willmer and Fricker (eds.) Topics in Functional Biology, 2. Stomata, Second Edition, Chapman and Hall Ltd., London, New York, 1996). Besides these rapid and transient changes, long term modulation of stomatal characteristics occurs which predominantly involve morphological aspects such as presence or absence of stomata in the upper and/or lower leaf epidermis, density of stomata in the leaf epidermices, or the size of the stomata. These features are determined both, by endogenous (genetic) and by exogenous (environmental) factors. Hints towards genetic control were obtained through the observation of a broad variation of the stomatal density between different species of the same genus, between varieties or cultivars of the same species, or in F1 hybrids. Genetic analysis revealed multigenic, oligogenic, or monogenic control and a high heritability of characters such as stomatal density or size (for review see Jones, *In Stomatal Function*, Eds. E. Zeiger, G.D. Farquhar and I.R. Cowan, pp. 431-443, Stanford University Press, Stanford 1987). In addition to this endogenous control, stomatal characteristics are also modulated through environmental effects. Thus, air humidity (e.g. Schürmann, 1959, *Flora* 147, 417-520), light intensity (e.g. Gay and Hurd, 1975, *New Phytol.* 75, 37-46; Schoch et al., 1980, *J. Exp. Bot.* 31, 1211-1216; Rahim and Fordham, 1991, *Ann. Bot.* 67, 167-171), and CO<sub>2</sub>-concentration (e.g. Woodward, 1987, *Nature* 327, 617-618; Woodward and Bazzaz, 1988, *J. Exp. Bot.* 39, 1771-1781; Goodfellow et al., 1997, *Tree Physiol.* 17, 291-299) were found to affect stomatal density. In several studies, stomatal density was found to be associated with plant yield (e.g. Walton, 1974, *Can J. Plant Sci.* 54, 749-754). Pima cotton varieties selected for high yield upon growth under conditions of high irradiance and artificial irrigation exhibit increased stomatal density associated with increased stomatal conductance and reduced leaf temperature (Cornish et al., 1991, *Plant Physiol.* 97, 484-489; Lu and Zeiger, 1994, *Physiol. Plant.* 92, 273-278; Lu et al., 1994, *Physiol. Plant.* 92, 266-272; Srivastava et al., 1995, *Plant Sci.* 19, 125-131). A similar relationship between stomatal conductance and yield was observed for a series of bread wheat varieties (Lu et al., 1998, *J. Exp. Bot.* 49, 453-460). According to these data, the modulation of stomatal characteristics are of high importance for the improvement of elite cultivars of crop plants. In the area of agriculture and forestry, a major aim is the continuous improvement of the crop plants with respect to higher yielding to provide sufficient food for the growing

global population and to ensure the supply of renewable resources. Traditionally, progress towards higher yielding varieties is attempted through breeding, a very labour and time consuming process to be conducted separately for every relevant plant species. Some progress has already been achieved through the application of genetic engineering to plants, i.e. the introduction and expression of recombinant nucleic acid molecules in plants. Such approaches are advantageous as they can usually be applied to many different plant species. In EP-A 0 511 979, for instance, the use of a procaryotic asparagine synthetase for expression in plant cells is described that, among other changes, leads to increased biomass production. WO 96/21737 describes yield increases in plants achieved through the expression of de- or non-regulated fructose-1,6-bisphosphatase through enhanced rate of photosynthesis. In WO 96/17069, the enhancement of biomass production in transgenic plants achieved through expression of a polyphosphate kinase from *E.coli* is described. In contrast to these cases, however, no means for a directed manipulation through genetic engineering of stomatal density or distribution in plants were hitherto available, due to the complete lack of knowledge about genes that are involved in the control of these stomatal characteristics.

Recently, an *Arabidopsis thaliana* mutant, R-558, has been isolated after chemical mutagenesis which shows a two to four-fold increase in the stomatal density of all aerial plant organs, in the leaves in particular and the occurrence of ca. 10% clustered stomata, i.e. stomata placed in direct contact to at least one other stomata (D. Berger, 1997, PhD Thesis Freie Universität Berlin). Besides a minor change in the length of the pedicelli, no other morphological changes were visible in the mutant plants. The form and size of the leaves as well as the structure of the mesophyll (number of cell layers in palisade and spongy parenchyma, form and size of the mesophyll cells) and the intercellular system (including the substomatal cavities) are unchanged. The increased stomatal density resulted in elevated transpiration (loss of H<sub>2</sub>O) and was associated with increased dry matter content in the leaves which in the wild type was ca. 3% and in the mutant ca. 7%. It was furthermore shown that the increased stomatal density in the R-558 mutant was associated with increased leaf fresh (+ 15%) and dry (+30%) weight, increased glucose (+70%), fructose (+65%), and protein (+50%) contents in leaves, and enhanced transpiration and CO<sub>2</sub>-assimilation (D. Berger, 1997, PhD Thesis, Freie

Universität Berlin) in comparison to the wild type. The mutation which caused the increased stomatal density has been mapped relative to a set of (molecular) genetic markers to a ca. 0.59 cM interval located on the top arm of chromosome 1 of *Arabidopsis thaliana* (D. Berger, 1997, PhD Thesis, Freie Universität Berlin). However, the regulation of stomatal density and distribution in plants is still not fully understood and means that can be used to manipulate stomatal characteristics such as density and distribution that may have applications in several aspects of agriculture were hitherto not available.

Thus, the technical problem underlying the present invention was to comply with the need for means and methods for modulating the stomatal density in plants. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the invention relates to a recombinant DNA molecule comprising

- (i) a nucleic acid molecule encoding a subtilisin-like serine protease or encoding a biologically active fragment of such a protein, selected from the group consisting of
  - (a) nucleic acid molecules comprising a nucleotide sequence encoding a protein comprising the amino acid sequence as given in SEQ ID NO: 2, 8, 10 or 12;
  - (b) nucleic acid molecules comprising a nucleotide sequence as given in SEQ ID NO: 1, 7, 9, or 11;
  - (c) nucleic acid molecules encoding a protein comprising at least the D region, H region, substrate binding site and/or S region of the subtilisin-like serine protease encoded by a nucleic acid molecule of (a) or (b); or
  - (d) nucleic acid molecules hybridizing with the complementary strand of a nucleic acid molecule as defined in any one of (a) to (c);
  - (e) nucleic acid molecules encoding a protein the amino acid sequence of which is at least 65% identical to the amino acid sequence encoded by a nucleic acid molecule of any one of (a) to (c);

- (f) nucleic acid molecules, the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (e); or
- (ii) a nucleic acid molecule encoding a mutant non-active or hyper-active form of or an antibody against the subtilisin-like serine protease encoded by a nucleic acid molecule of (i); or
- (iii) a nucleic acid molecule which specifically hybridizes with a nucleic acid molecule of (i) or the complementary strand thereof.

The present invention is based on the identification of a new class of genes represented by *SDD1* from *Arabidopsis thaliana* which share common structural motifs, see infra. In one aspect, these genes are preferably involved in the control of stomatal density and/or distribution. The *SDD1* gene is mutated in the *Arabidopsis thaliana* mutant R-558; see Examples 1 to 3. Computer-assisted amino acid sequence analysis of the protein encoded by this gene revealed that it belongs to a family of subtilisin-like serine proteases; see Example 4. Further representatives of this new class of genes have been cloned from potato; see Example 6.

The terms "subtilisin, like-serine protease" and "subtilase" are used interchangeable herein and mean a specific class of serine proteases, called subtilisins or dibasic processing endoproteases. In subtilisins, four regions form the catalytic triad and the substrate binding site and are most highly conserved among subtilisins; see also Example 4 and Figure 7. In the context of the present invention, subtilisin-like serine proteases also mean such proteins which show a homology of at least 65% to the sequence shown in SEQ ID NOs: 2, 8, 10 or 12. In the context of the present invention, the term "subtilisin-like serin protease" preferably is understood to mean proteins comprising one or several of the characteristic motifs depicted in SEQ ID NOS: 13 to 37; see infra.

The substrate binding site preferably comprises the motif VICAA (SEQ ID NO: 38), more preferably the motif CAAGN (SEQ ID NO: 39), in particular the motif AAGNN (SEQ ID NO: 40) and most preferably the amino acid motif VICAAGNNG (SEQ ID NO: 41).

In another preferred embodiment, the nucleic acid molecule of the present invention encodes a protein described above with one or more of the following amino acid sequence motifs: SYHSA (SEQ ID NO: 49), GLSPT (SEQ ID NO: 50), WLKSG (SEQ ID NO: 51), FNSSS (SEQ ID NO: 52), ASTAG (SEQ ID NO: 53), AAMDV (SEQ ID NO: 54), WIATI (SEQ ID NO: 55), GPSGL (SEQ ID NO: 56), IAALLH (SEQ ID NO: 57), KPIMD (SEQ ID NO: 58), VSCHD (SEQ ID NO: 59), YPSIS (SEQ ID NO: 60), SLSYR (SEQ ID NO: 61).

In a further preferred embodiment the D, H and/or S region of the subtilase of the present invention comprise one or several of the following characteristic motifs:

D region:

IIGVL (SEQ ID NO: 42) or GVLDT (SEQ ID NO: 43)

H region:

THTAST (SEQ ID NO: 44) or S-RDS (SEQ ID NO: 45) or RDS-G (SEQ ID NO: 46)

S region:

HVSGI (SEQ ID NO: 47) or FTV-SGT (SEQ ID NO: 48)

While a function of such proteins in the regulation of stomatal density in plants was hitherto unknown, the present invention for the first time provides evidence that the described nucleic acid molecules encode proteins that are involved in controlling the density and the distribution of stomata in plants; see Examples 3 and 7. Furthermore, it is shown that plants lacking or overexpressing such proteins show altered morphological and physiological features of high agronomic importance.

Thus, the present invention for the first time clearly establishes that stomatal characteristics such as density and distribution can be specifically modulated through the application of genetic engineering techniques and provides extremely useful tools for example to:

- (i) generate plants with increased stomatal density and consequently with enhanced CO<sub>2</sub> assimilation, reduced leaf temperature, enhanced organ such as leaf fresh and dry weight, and enhanced sugar and protein contents in organs such as leaves;
- (ii) generate plants with decreased stomatal density and consequently with reduced water loss and thus lower water consumption;



- (iii) counteract environmental changes such as raises in atmospheric CO<sub>2</sub> levels, temperature and irradiation that would cause changes in stomatal density to sub- or supra-optimal levels;

In general a nucleic acid molecule encoding a subtilisin-like serine protease can be derived from any material source, for example, from any organism, preferably plants possessing such molecules, preferably from monocotyledonous or dicotyledonous plants, in particular from any plant of interest in agriculture, horticulture or wood culture, such as crop plants, namely those of the family Poaceae, any starch producing plants, such as potato, maniok, rice, wheat, corn, barley, oat, leguminous plants, oil producing plants, such as oilseed rape, soja, sunflower, linenseed, etc., plants using polypeptide as storage substances, such as soybean, plants using sucrose as storage substance, such as sugar beet or sugar cane, trees, ornamental plants etc. or plants belonging to the family Gramineae.

Furthermore, nucleic acid molecules can be used in accordance with the present invention hybridizing to the above-described nucleic acid molecules and encoding subtilisin-like serine protease. Such nucleic acid molecules can be isolated, e.g., from libraries, such as cDNA or genomic libraries by techniques well known in the art. For example, hybridizing nucleic acid molecules can be identified and isolated by using the above-described nucleic acid molecules or fragments thereof or complements thereof as probes to screen libraries by hybridizing with said molecules according to standard techniques. Possible is also the isolation of such nucleic acid molecules by applying the polymerase chain reaction (PCR) using as primers oligonucleotides derived from the above-described nucleic acid molecules. Nucleic acid molecules which hybridize with any of the aforementioned nucleic acid molecules also include fragments, derivatives and allelic variants of the above-described nucleic acid molecules that encode subtilisin-like serine proteases or biologically active fragments thereof. Fragments are understood to be parts of nucleic acid molecules long enough to encode the described protein or a fragment thereof having the biological activity as defined above. Preferably, said fragment comprises at least one region of subtilisin-like serine protease as defined in section (i) (c) supra.

The term "derivative" means in this context that the nucleotide sequence of these nucleic acid molecules differs from the sequences of the above-described nucleic acid molecules in one or more nucleotide positions and are highly homologous to said nucleic acid molecules. Homology is understood to refer to a sequence identity of at least 50 %, preferably 65% identity, particularly an identity of at least 70 % or 75%, preferably more than 80 % and still more preferably more than 90 % or 95% identity. The deviations from the sequences of the nucleic acid molecules described above can, for example, be the result of nucleotide substitution(s), deletion(s), addition(s), insertion(s) and/or recombination(s) either alone or in combination, that may naturally occur or be produced via recombinant DNA techniques well known in the art; see for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Homology further means that the respective nucleic acid molecules or encoded proteins are functionally and/or structurally equivalent. The nucleic acid molecules that are homologous to the nucleic acid molecules described above and that are derivatives of said nucleic acid molecules are, for example, variations of said nucleic acid molecules which represent modifications having the same biological function, in particular encoding proteins with the same or substantially the same biological activity as defined herein. They may be naturally occurring variations, such as subtilisin-like serine protease encoding sequences from other prokaryotes and eukaryotes, respectively, or mutations. These mutations may occur naturally or may be obtained by mutagenesis techniques; see supra. The allelic variations may be naturally occurring allelic variants as well as synthetically produced or genetically engineered variants; see supra. For example, the amino acid sequences of plant subtilisin-like serine proteases share significant similarities with bacterial, yeast and mammalian subtilisin-like serine protease; see Example 4. In addition, nucleic acid molecules can be employed in accordance with the present invention that encode homologs or analogs of the above described subtilisin-like serine protease but where otherwise unrelated to those proteins. Such proteins that do not display significant homologies to common subtilisin-like serine protease can be identified by a person skilled in the art using techniques

well known in the art, for example, via complementation of mutant genes, for example, in corresponding mutant plants; see Example 3.

In a further embodiment the term derivative encompasses a nucleotide sequence coding for a protein derived from any one of SEQ ID Nos. 2, 8, 10 or 12 which exhibits a degree of homology, i.e. identity to the protein depicted under SEQ ID Nos. 2, 8, 10 or 12 of at least 60%, in particular an homology of at least 70%, preferably more than 80% and still more preferably a homology of more than 90% and particularly preferred of more than 95% and which exhibits at least one, more preferably at least three, even more preferably of at least five, in particular at least ten and particularly preferred of at least twenty of the peptide motifs selected from the group consisting of

- a) QTYIV, (SEQ ID NO: 13),
- b) IVQLH, (SEQ ID NO: 14),
- c) SSRLL, (SEQ ID NO: 15),
- d) QTTYS, (SEQ ID NO: 16),
- e) SSSCN, (SEQ ID NO: 17),
- f) VLGNG, (SEQ ID NO: 18),
- g) GAHIA, (SEQ ID NO: 19),
- h) FRAME, (SEQ ID NO: 20),
- i) VICAA, (SEQ ID NO: 21),
- j) AAGNN, (SEQ ID NO: 22),
- k) SSVAN, (SEQ ID NO: 23),
- l) YGESL, (SEQ ID NO: 24),
- m) GSEFC, (SEQ ID NO: 25),
- n) CLRGS, (SEQ ID NO: 26),
- o) RGVNG, (SEQ ID NO: 27),
- p) PATLIG, (SEQ ID NO: 28),
- q) IFGGT, (SEQ ID NO: 29),
- r) PQNLG, (SEQ ID NO: 30),
- s) VNFTV, (SEQ ID NO: 31),
- t) HVSGI, (SEQ ID NO: 32),
- u) GFSLN, (SEQ ID NO: 33),
- v) RRVTN, (SEQ ID NO: 34),
- w) PNSIY, (SEQ ID NO: 35),
- x) LSYRV, (SEQ ID NO: 36), and
- y) SPISV. (SEQ ID NO: 37)

The proteins encoded by the various derivatives, variants, homologs or analogs of the above-described nucleic acid molecules may share specific common characteristics, such as molecular weight, immunological reactivity, conformation, etc., as well as physical properties, such as electrophoretic mobility, chromatographic behavior, sedimentation coefficients, pH optimum, temperature

optimum, stability, solubility, spectroscopic properties, etc. All these nucleic acid molecules and derivatives can be employed in accordance with the present invention as long as the biological activity of the encoded protein remains substantially unaffected in kind, namely that the protein is capable of modulating stomata density in plants. Any one of the above described nucleic acid molecules, in particular those that represent hyper-active mutant forms of subtilisin-like serine proteases are particular suitable for overexpression in transgenic plants. These transgenic plants may either possess an endogenous functional subtilisin-like serine protease or they may lack the corresponding genes, e.g. due to mutation.

The nucleic acid molecules mentioned in section (ii) and (iii) are particular useful for the suppression of genes encoding subtilisin-like serine proteases in plants. Hence, in one embodiment said nucleic acid molecules are preferably of at least 50 nucleotides in length hybridizing specifically with a nucleic acid molecule as described above or with a complementary strand thereof. Specific hybridization occurs preferably under stringent conditions and implies no or very little cross-hybridization with nucleotide sequences encoding no or substantially different proteins. In particular stringent conditions mean, e.g., the use of an aqueous solution of 1% BSA, 1mM EDTA, 0.5 M NaHPO<sub>4</sub> pH7.2, 7% SDS and incubation at 65°C. Preferably, stringent hybridization is obtained under the following conditions:

Hybridization buffer:

2 x SSC; 10 x Denhardt's solution (Ficoll 400 + PEG + BSA; ratio 1:1:1); 0.1% SDS; 5mM EDTA; 50 mM Na<sub>2</sub>HPO<sub>4</sub>; 250 µg/ml herring sperm DNA; 50 µ/ml tRNA; or

0.25M sodium phosphate buffer pH 7.2; 1mM EDTA; 7% SDS

Hybridization temperature: T=65 to 68°C

Washing buffer: 0.2 x SSC; 0.1% SDS

Washing temperature: T=68°C

Such nucleic acid molecules may be used as probes and/or for the control of gene expression. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary in length. Preferred are nucleic acid probes of 50 nucleotides or more in length. Of course, it may also be

appropriate to use nucleic acids of up to 100 and more nucleotides in length. The nucleic acid probes of the invention are useful for various applications. On the one hand, they may be used as PCR primers for amplification of nucleic acid sequences according to the invention. Another application is the use as a hybridization probe to identify nucleic acid molecules hybridizing with a nucleic acid molecule of the invention by homology screening of genomic DNA or cDNA libraries. Nucleic acid molecules according to this preferred embodiment of the invention which are complementary to a nucleic acid molecule as described above can be used for repression of expression of a subtilisin-like serine protease encoding gene, for example due to an antisense or triple helix effect or for the construction of appropriate ribozymes (see, e.g., EP-A1 0 291 533, EP-A1 0 321 201, EP-A2 0 360 257) which specifically cleave the (pre)-mRNA of a gene comprising a nucleic acid molecule of the invention or part thereof. Selection of appropriate target sites and corresponding ribozymes can be done as described, for example, in Steinecke, Ribozymes, Methods in Cell Biology 50, Galbraith et al. eds Academic Press, Inc. (1995), 449-460. Furthermore, the person skilled in the art is well aware that it is also possible to label such a nucleic acid probe with an appropriate marker for specific applications, such as for the detection of the presence of a nucleic acid molecule of the invention in a sample derived from an organism, in particular plants.

The above described nucleic acid molecules may either be DNA or RNA or a hybrid thereof. Furthermore, said nucleic acid molecule may contain, for example, thioester bonds and/or nucleotide analogues, commonly used in oligonucleotide anti-sense approaches. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell.

Furthermore, nucleic acid molecules encoding antibodies specifically recognizing a subtilisin-like serine protease or parts, i.e. specific fragments or epitopes, of such a protein can be used for inhibiting the activity of the protein in plants. These antibodies can be monoclonal antibodies, polyclonal antibodies or synthetic antibodies as well as fragments of antibodies, such as Fab, Fv or scFv fragments etc. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to

spleen cells derived from immunized mammals. Furthermore, antibodies or fragments thereof to the aforementioned peptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. These antibodies can be used, for example, for the immunoprecipitation and immunolocalization of proteins according to the invention as well as for the monitoring of the synthesis of such proteins, for example, in recombinant organisms, and for the identification of compounds interacting with the protein according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies selections, yielding a high increment of affinity from a single library of phage antibodies which bind to an epitope of the protein of the invention (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmberg, J. Immunol. Methods 183 (1995), 7-13). Expression of antibodies or antibody-like molecules in plants can be achieved by methods well known in the art, for example, full-size antibodies (Düring, Plant. Mol. Biol. 15 (1990), 281-293; Hiatt, Nature 342 (1989), 469-470; Voss, Mol. Breeding 1 (1995), 39-50), Fab-fragments (De Neve, Transgenic Res. 2 (1993), 227-237), scFvs (Owen, Bio/Technology 10 (1992), 790-794; Zimmermann, Mol. Breeding 4 (1998), 369-379; Tavladoraki, Nature 366 (1993), 469-472) and dAbs (Benvenuto, Plant Mol. Biol. 17 (1991), 865-874) have been successfully expressed in Tobacco, Potato (Schouten, FEBS Lett. 415 (1997), 235-241) or *Arabidopsis*, reaching expression levels as high as 6.8% of the total protein (Fiedler, Immunotechnology 3 (1997), 205-216).

In addition, nucleic acid molecules encoding mutant forms of a subtilisin-like serine protease can be used to interfere with the activity of the wild type protein. Such mutant forms preferably have lost their biological activity as defined above and may be derived from the corresponding subtilisin-like serine protease by way of amino acid deletion(s), substitution(s), and/or additions in the amino acid sequence of the protein. As mentioned above, mutant forms of subtilisin-like serine proteases also encompass hyper-active mutant forms of such proteins which display, e.g. an increased substrate affinity and/or higher substrate turnover of the same. Furthermore, such hyper-active forms may be more stable in the cell due to the incorporation of amino acids that stabilize proteins in the cellular environment.

These mutant forms may be naturally occurring or genetically engineered mutants; see also supra.

The recombinant DNA molecule of the invention preferably comprises regulatory sequences allowing for the expression the nucleic acid molecules in plants. Preferably, said regulatory elements comprise a promoter active in plant cells. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in plant cells are well known to those skilled in the art.

These regulatory elements may be homologous or preferably heterologous with respect to the nucleic acid molecule to be expressed and/or with respect to the plant species to be transformed. For example, a preferred regulatory element that can be employed in accordance with the present invention is the SDD1 promoter region as depicted in SEQ ID NO: 5 or part thereof.

Preferably, the promoter region of the SDD1 gene comprising SEQ ID NO: 6 is employed, which corresponds to the nucleotide sequence of SEQ ID NO: 5 starting at position 839. GUS expression studies show that the promoter of SDD1 of *Arabidopsis thaliana* in tissues having mitotic activity shows a high activity. For example, a very strong GUS expression can be found in developing stomata and in primordials, but also a weaker expression in lateral roots. By way of computer-assisted studies different domains could be identified which possibly are responsible for the expression pattern of this promoter. On the one hand, a domain was identified which allows expression in roots, on the other hand several characteristic motifs were identified which are termed Dof-motifs (see, e.g., Yanagisawa and Schmidt, Plant J. 17 (1999), 209-214) and which in the present case possibly allow for an expression in guard cells. These motifs have, e.g., been described in German patent application DE 19904754.5. It is assumed that a deletion of the domain, which possibly mediates the expression in roots and which is located within the first 400bp of SEQ ID NO: 6, advantageously changes the specificity of the promoter. For this reason preferred embodiments of the invention use promoter fragments comprising at the 5'-region a deletion of at least 400-450bp or of 450-600bp or at the most 900bp.

It is possible for the person skilled in the art to isolate with the help of the coding and regulatory sequences of the invention corresponding genes from other species, for example, potato, tomato, barley, wheat, oat, rye, rice, corn, soja, etc. This can be done by conventional techniques known in the art, for example, by using the regulatory sequences depicted in SEQ ID NO: 6 as a hybridization probe or by designing appropriate PCR primers. It is then possible to isolate the corresponding promoter region by conventional techniques and test it for its expression pattern. For this purpose, it is, for instance, possible to fuse the promoter to a reporter gene, such as GUS, luciferase or green fluorescent protein (GFP) and assess the expression of the reporter gene in transgenic plants.

For example the promoters from the two SDD1 homologs from *Solanum tuberosum* described in Example 6 can be isolated by conventional means. Genomic clones can be amplified, e.g., fragments via long template PCR (employing for example the EXPAND kit, Boehringer Mannheim), using an upstream oriented SDD1 specific primer and a primer to the Lambda left or right arm sequence. The amplified fragment is sequenced via primer walking until several kb upstream from the transcription start point have been reached, if present on the clone, preferably more than 3 kb. Within the cloned genomic sequence, the transcription start site is determined by standard procedures well known to the person skilled in the art, such as 5'-RACE, primer extension or S1 mapping. To define cis-regulatory elements upstream of the transcription start site (i.e. within the putative promoter region), the respective region is fused to marker genes such as genes encoding GUS or GFP, and 5' deletion derivatives of these construct are generated. They are transformed into suitable plant material, and the expression of the marker gene depending on the remaining upstream sequence (putative promoter) is determined. These techniques are well known to the person skilled in the art.

The regulatory sequences so identified may differ at one or more positions from the above-mentioned regulatory sequence but still have the same specificity, namely they comprise the same or similar sequence motifs, preferably 6 to 10 nucleotides in length, responsible for the above described expression pattern. Preferably such regulatory sequences hybridize to one of the above-mentioned regulatory sequences, most preferably under stringent conditions. Particularly



preferred are regulatory sequences which share at least 85%, more preferably 90-95%, and most preferably 96-99% sequence identity with one of the above-mentioned regulatory sequences and have the same or substantially the same specificity. Particularly preferred are the regulatory sequences that comprise the above mentioned motifs which allow for an expression in guard cells. Such regulatory sequences also comprise those which are altered, for example by nucleotide deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) known in the art either alone or in combination in comparison to the above-described nucleotide sequence. Methods for introducing such modifications in the nucleotide sequence of the regulatory sequences of the invention are well known to the person skilled in the art. It is also immediately evident to the person skilled in the art that further regulatory elements may be added to the regulatory sequences of the invention. For example, transcriptional enhancers and/or sequences which allow for induced expression of the regulatory sequences of the invention may be employed.

The possibility exists to modify the regulatory sequences as described above or sequence motifs thereof by, e.g., nucleotide replacements which do not affect the overall structure or binding motif of the regulatory sequence so that it remains capable of conferring the gene expression pattern as described above. Such regulatory sequences may be derived from subtilase genes of potato although other plants may be suitable sources for such regulatory sequences as well. Furthermore, the nucleotide sequences can be compared using appropriate computer programs known in the art such as BLAST, which stands for Basic Local Alignment Search Tool (Altschul, 1997; Altschul, J. Mol. Evol. 36 (1993), 290-390; Altschul, J. Mol. Biol. 215 (1990); 403-410), to search for local sequence alignments. BLAST produces alignments of nucleotide sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues.

In general, regulatory elements employed in accordance with the present invention comprise a promoter active in plant cells. To obtain expression in all tissues of a transgenic plant, preferably constitutive promoters are used, such as the 35S promoter of CaMV (Odell, Nature 313 (1985), 810-812) or promoters of the

polyubiquitin genes of maize (Christensen, Plant Mol. Biol. 18 (1982), 675-689). In order to achieve expression in specific tissues of a transgenic plant it is possible to use tissue specific promoters (see, e.g., Stockhaus, EMBO J. 8 (1989), 2245-2251). Known are also promoters which are specifically active in tubers of potatoes or in seeds of different plants species, such as maize, Vicia, wheat, barley etc. Inducible promoters may be used in order to be able to exactly control expression. An example for inducible promoters are the promoters of genes encoding heat shock proteins. Further useful promoters are described in the prior art; see, e.g.:

- a) inducible promoters:  
described in WO 93/21334 (=alcA/alcR system), WO 90/08826, WO 96/37609.
- b) promoters active in photosynthetically active tissue:  
the ST-LS1 promoter (Stockhaus et al., Proc. Natl. Acad. Sci. USA 84 (1987), 7943-7947; Stockhaus et al., EMBO J. 8 (1989), 2445-2451), the Ca/b promoter (see e.g. US-A-5,656,496; US-A-5,639,952; Bansal et al., Proc. Natl. Acad. Sci. USA 89 (1992), 3654-3658) and the Rubisco SSU promoter (see e.g. US-A-5,034,322; US-A-4,962,028) as well as the STL1 promoter (Eckes et al., Mol. Gen. Genet. 205 (1986), 14-22).
- c) promoters and cis-active elements mediating expression in guard cells:
  - DE 19904754.5
  - DE 4207358
  - truncated AGPase promoter (Müller-Röber et al., Plant Cell 6 (1994), 601-612)
  - Rha1 promoter (Terryn et al., Plant Cell 5 (1993), 1761-1769)
- d) promoters mediating expression in meristematic tissue:
  - wheat histone H4 promoter (Bilgin et al., Plant Science 143 (1999), 35-44)
  - rice PCNA promoter (Kosugi et al., Plant J. 7 (1995), 877-886)
  - wheat histone H2B promoter (Yang et al., Plant Mol. Biol. 28 (1994), 155-172)
  - cyc07-promoter (Ito et al., Plant Mol. Biol. 24 (1994), 863-878).

Also microspore-specific regulatory elements and their uses have been described (WO96/16182). Furthermore, the chemically inducible Tet-system may be employed (Gatz, Mol. Gen. Genet. 227 (1991); 229-237). Further suitable promoters are

known to the person skilled in the art and are described, e.g., in Ward (Plant Mol. Biol. 22 (1993), 361-366). The regulatory elements may further comprise transcriptional and/or translational enhancers functional in plants cells. A plant translational enhancer often used is, e.g., the CaMV omega sequences and/or the inclusion of an intron (Intron-1 from the Shrunk gene of maize, for example) that has been shown to increase expression levels by up to 100-fold. (Maiti, Transgenic Research 6 (1997), 143-156; Ni, Plant Journal 7 (1995), 661-676). Furthermore, the regulatory elements may include transcription termination signals, such as a poly-A signal, which lead to the addition of a poly A tail to the transcript which may improve its stability. The termination signals usually employed are from the Nopaline Synthase gene or from the CaMV 35S RNA gene.

In a preferred embodiment of the recombinant DNA molecule of the invention, the subtilisin-like serine protease is derived from plants. Preferably, said plants are monocotyledonous or dicotyledonous plants such as those mentioned hereinbefore. A particular preferred embodiment of said plant is Arabidopsis.

The present invention also relates to vectors, particularly plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering that contain at least one recombinant DNA molecule according to the invention. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, the recombinant DNA molecules and vectors of the invention can be reconstituted into liposomes for delivery to target cells.

Advantageously the above-described vectors of the invention comprises a selectable and/or scorable marker. Selectable marker genes useful for the selection of transformed plant cells, callus, plant tissue and plants are well known to those skilled in the art and comprise, for example, antimetabolite resistance as the basis of selection for dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149); npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella,

EMBO J. 2 (1983), 987-995) and hpt, which confers resistance to hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from *Aspergillus terreus* which confers resistance to Blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338). Useful scorable marker are also known to those skilled in the art and are commercially available. Advantageously, said marker is a gene encoding luciferase (Giacomin, Pl. Sci. 116 (1996), 59-72; Scikantha, J. Bact. 178 (1996), 121), green fluorescent protein (Gerdes, FEBS Lett. 389 (1996), 44-47) or  $\beta$ -glucuronidase (Jefferson, EMBO J. 6 (1987), 3901-3907). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a vector of the invention. As described above, various selectable markers can be employed in accordance with the present invention. Advantageously, selectable markers may be used that are suitable for direct selection of transformed plants, for example, the phosphinothricin-N-acetyltransferase gene the gene product of which detoxifies the herbicide L-phosphinothricin (glufosinate or BASTA); see, e.g., De Block, EMBO J. 6 (1987), 2513-2518 and Dröge, Planta 187 (1992), 142-151.

The present invention, also relates to host cells comprising a recombinant DNA molecule or vector of the invention. Host cells include prokaryotic and eukaryotic cells such as *E. coli* and yeast, respectively.

The recombinant DNA molecules according to the invention are in particular useful for the genetic manipulation of plant cells, plant tissue and plants in order to obtain plants with modified, preferably with improved or useful phenotypes as described above. Thus, the present invention relates to a method for the production of transgenic plants with altered stomata characteristics compared to wild type

plants comprising the introduction of a recombinant DNA molecule of the invention into the genome of a plant, plant cell or plant tissue.

Methods for the introduction of foreign DNA into plants as well as the selection and regeneration of transgenic plants from plant cells and plant tissue are also well known in the art. These include, for example, the transformation of plant cells, plant tissue or plants with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*, the fusion of protoplasts, direct gene transfer (see, e.g., EP-A 164 575), injection, electroporation, biolistic methods like particle bombardment and other methods known in the art. The vectors used in the method of the invention may contain further functional elements, for example "left border"- and "right border"-sequences of the T-DNA of *Agrobacterium* which allow for stable integration into the plant genome. Furthermore, methods and vectors are known to the person skilled in the art which permit the generation of marker free transgenic plants, i.e. the selectable or scorable marker gene is lost at a certain stage of plant development or plant breeding. This can be achieved by, for example cotransformation (Lyznik, *Plant Mol. Biol.* 13 (1989), 151-161; Peng, *Plant Mol. Biol.* 27 (1995), 91-104) and/or by using systems which utilize enzymes capable of promoting homologous recombination in plants (see, e.g., WO97/08331; Bayley, *Plant Mol. Biol.* 18 (1992), 353-361; Lloyd, *Mol. Gen. Genet.* 242 (1994), 653-657; Maeser, *Mol. Gen. Genet.* 230 (1991), 170-176; Onouchi, *Nucl. Acids Res.* 19 (1991), 6373-6378). Methods for the preparation of appropriate vectors are described by, e.g., Sambrook (*Molecular Cloning; A Laboratory Manual*, 2nd Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Suitable strains of *Agrobacterium tumefaciens* and vectors as well as transformation of *Agrobacteria* and appropriate growth and selection media are well known to those skilled in the art and are described in the prior art (GV3101 (pMK90RK), Koncz, *Mol. Gen. Genet.* 204 (1986), 383-396; C58C1 (pGV 3850kan), Deblaere, *Nucl. Acid Res.* 13 (1985), 4777; Bevan, *Nucleic. Acid Res.* 12(1984), 8711; Koncz, *Proc. Natl. Acad. Sci. USA* 86 (1989), 8467-8471; Koncz, *Plant Mol. Biol.* 20 (1992), 963-976; Koncz, *Specialized vectors for gene tagging and expression studies*. In: *Plant Molecular Biology Manual Vol 2*, Gelvin and Schilperoort (Eds.), Dordrecht, The Netherlands: Kluwer Academic Publ. (1994), 1-22; EP-A-120 516; Hoekema: *The Binary Plant Vector System*, Offsetdrukkerij

Kanters B.V., Alblasserdam (1985), Chapter V, Fraley, Crit. Rev. Plant. Sci., 4, 1-46; An, EMBO J. 4 (1985), 277-287). Although the use of *Agrobacterium tumefaciens* is preferred in the method of the invention, other *Agrobacterium* strains, such as *Agrobacterium rhizogenes*, may be used, for example if a phenotype conferred by said strain is desired.

Methods for the transformation using biolistic methods are well known to the person skilled in the art; see, e.g., Wan, Plant Physiol. 104 (1994), 37-48; Vasil, Bio/Technology 11 (1993), 1553-1558 and Christou (1996) Trends in Plant Science 1, 423-431. Microinjection can be performed as described in Potrykus and Spangenberg (eds.), Gene Transfer To Plants. Springer Verlag, Berlin, NY (1995).

The transformation of most dicotyledonous plants is possible with the methods described above. But also for the transformation of monocotyledonous plants several successful transformation techniques have been developed. These include the transformation using biolistic methods as, e.g., described above as well as protoplast transformation, electroporation of partially permeabilized cells, introduction of DNA using glass fibers, etc. Transgenic plant tissue and plants can be regenerated by methods well known in the art. There are various references in the relevant literature dealing specifically with the transformation of maize (cf. e.g. WO95/06128, EP 0 513 849; EP 0 465 875). In EP 292 435 a method is described by means of which fertile plants may be obtained starting from mucousless, friable granulous maize callus. In this context it was furthermore observed by Shillito et al., Bio/Technology 7 (1989), 581 that for regenerating fertile plants it is necessary to start from callus-suspension cultures from which a culture of dividing protoplasts can be produced which is capable to regenerate to plants. After an in vitro cultivation period of 7 to 8 months Shillito et al. obtain plants with viable descendants which, however, exhibited abnormalities in morphology and reproductivity.

Prioli and Söndahl, Bio/Technology 7 (1989), 589 have described how to regenerate and to obtain fertile plants from maize protoplasts of the Cateto maize inbreed Cat 100-1. The authors assume that the regeneration of protoplasts to fertile plants depends on a number of various factors such as the genotype, the physiological state of the donor-cell and the cultivation conditions. With regard to rice various transformation methods can be applied, e.g. the transformation by agrobacterium-mediated gene transfer (Hiei et al., Plant J. 6 (1994), 271-282; Hiei et al., Plant Mol.

Biol. 35 (1997), 205-218; Park et al., J. Plant Biol. 38 (1995), 365-371), protoplast transformation (Datta in "Gene transfer to plants", I. Potrykus, G. Spangenberg (Eds.), Springer-Verlag Berlin Heidelberg (1995), pages 66-75; Datta et al., Plant Mol. Biol. 20 (1992), 619-629; Sadasivam et al., Plant Cell Rep. 13 (1994), 394-396), the biolistic approach (Li et al., Plant Cell Rep. 12 (1993), 250-255; Cao et al., Plant Cell Rep. 11 (1992), 586-591; Christou, Plant Mol. Biol. (1997), 197-203) and electroporation (Xu et al., in "Gene transfer to plants", I. Potrykus, G. Spangenberg (Eds.), Springer-Verlag Berlin Heidelberg (1995), pages 201-208.

Once the introduced DNA has been integrated in the genome of the plant cell, it usually continues to be stable there and also remains with the descendants of the originally transformed cell. It usually contains a selectable marker which confers resistance against biozides or against an antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricine etc. to the transformed plant cells. The individually selected marker should therefore allow for a selection of transformed cells against cells lacking the introduced DNA.

The transformed cells grow in the usual way within the plant (see also McCormick et al., Plant Cell Rep. 5 (1986), 81-84). The resulting plants can be cultivated in the usual way and cross-bred with plants having the same transformed genetic heritage or another genetic heritage. The resulting hybrid individuals have the corresponding phenotypic properties.

Two or more generations should be grown in order to ensure whether the phenotypic feature is kept stably and whether it is transferred. Furthermore, seeds should be harvested in order to ensure that the corresponding phenotype or other properties will remain.

In general, the plants, plant cells and plant tissue which can be modified with a recombinant DNA molecule or vector according to the invention can be derived from any desired plant species. They can be monocotyledonous plants or dicotyledonous plants, preferably they belong to plant species of interest in agriculture, wood culture or horticulture, such as crop plants (e.g. maize, rice, barley, wheat, rye, oats etc.), vegetable plants such as tomato, melon, banana, chicoree, salad, cabbage or potato, tobacco, alfalfa, clover, oil producing plants (e.g. oilseed rape, sunflower, peanut, soybean, etc.), cotton, sugar beet, linseed, flax, millet, hemp, sugar cane,

leguminous plants (e.g. beans, peas etc.), wood producing plants, preferably trees, etc.

Thus, the present invention relates also to transgenic plant cells which contain a nucleic acid molecule as defined above or a recombinant DNA molecule or vector according to the invention wherein the nucleic acid molecule is foreign to the transgenic plant cell.

By "foreign" it is meant that the nucleic acid molecule is either heterologous with respect to the plant cell, this means derived from a cell or organism with a different genomic background, or is homologous with respect to the plant cell but located in a different genomic environment than the naturally occurring counterpart of said nucleic acid molecule. This means that, if the nucleic acid molecule is homologous with respect to the plant cell, it is not located in its natural location in the genome of said plant cell when stably integrated into the genome, in particular it is surrounded by different genes. In this case the nucleic acid molecule may be either under the control of its own promoter or under the control of a heterologous promoter. The nucleic acid molecule, vector or recombinant DNA molecule according to the invention which is present in the plant cell may either be integrated into the genome of the plant cell or it may be maintained in some form extrachromosomally.

In one aspect the present invention relates to a transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of the invention or a vector of the present invention or obtainable according to the method of the invention wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild-type plants.

The term "increase in activity" in the context of the present invention is understood to mean an increase in the expression of endogenous genes coding for a protein of the invention and/or an increase of the amount of the protein of the invention in the cells.

The increase in expression can for example be determined by measuring the amount of transcripts encoding the protein of the invention, e.g., by Northern-blot analysis, preferably by the more sensitive NASBA method (as e.g. described by



Leone et al., *Journal of Virological Methods* 66 (1997), 19-27; Leone et al., *Nucleic Acid Res.* 26 (1998), 2150-2155; Nakahara et al., *Nucleic Acid Res.* 26 (1998), 1854-1856) or by RT-PCR. Preferably, an increase in this context means an increase of the amount of transcripts encoding subtilases as compared to corresponding cells which are not genetically modified by at least 5%, more preferably by at least 20%, in particular by at least 50%, and most preferably by at least 400%.

Preferably, the increased expression or activity of subtilisin-like serine proteases in transgenic plants results in decreased stomata density, see, e.g., Example 7.

The increase of the amount of the protein of the invention can for example be determined by Western-blot analysis. Preferably, an increase in this context means an increase of the amount of the protein of the invention as compared to corresponding cells which are not genetically modified by at least 5%, more preferably by at least 20%, in particular by at least 50%, and most preferably by at least 400%.

Alternatively, a plant cell having a nucleic acid molecule encoding a subtilisin-like serine protease present in its genome can be used and modified such that said plant cell expresses the endogenous gene corresponding to this nucleic acid molecules under the control of heterologous promoter and/or enhancer elements. The introduction of the heterologous promoter and mentioned elements which do not naturally control the expression of a nucleic acid molecule encoding a subtilisin-like serine protease using, e.g., gene targeting vectors can be done according to standard methods, see *supra* and, e.g., Hayashi, *Science* 258 (1992), 1350-1353; Fritze and Walden, *Gene activation by T-DNA tagging. In Methods in Molecular biology* 44 (Gartland, K.M.A. and Davey, M.R., eds). Totowa: Human Press (1995), 281-294) or transposon tagging (Chandlee, *Physiologia Plantarum* 78 (1990), 105-115). Suitable promoters and other regulatory elements such as enhancers include those mentioned hereinbefore.

Furthermore, the present invention relates to transgenic plants or plant tissue comprising plant cells of the invention or obtainable by the above described method. Preferably, the transgenic plant of the invention displays a decreased stomata density, lower conductance of stomata and/or the water consumption is lowered compared to wild type plants.

Methods for determining stomatal density, leaf conductance and water consumption comprise the following:

- Method for determining stomatal density by means of taking copies using clear nail varnish as described in Sachs et al., *Annals of Botany* 71 (1993), 237-243.
- Method for determining conductance as described in Muschak et al., *Photosynthetica* 33 (1997), 455-465.
- Methods for determining water consumption are known to the person skilled in the art.

Preferably, the transgenic plant of the invention displays one or more of the following phenotypes:

- a) stomatal density: reduced by at least 2%, preferably by at least 5%, more preferably by at least 10%, most preferably by at least 30%;
- b) conductance reduced by at least 2%, preferably by at least 5%, more preferably by at least 10%, most preferably by at least 25%;
- c) water consumption reduced by at least 1%, preferably by at least 3%, more preferably by at least 5%, most preferably by at least 10%;

as compared to a corresponding wild type plant.

In another aspect, the present invention relates to a transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of the invention or part thereof, a vector of the present invention or obtainable according to the method of the invention, wherein the presence, transcription and/or expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.

Usually the activity will be reduced by at least 10%, preferably by at least 30%, more preferably by at least 70%, most preferably by at least 100%. Methods of how to determine a decrease in activity as well as the definition of the term "activity" have been mentioned in the above. As it appears to be the case that, even minor changes in the amount of expression can have some effect on the phenotype of the plant methods such as NASBA analysis and RT-PCR which are considerably more sensitive in place of the Northern-blot analysis are employed for the analysis of the transgenic plant.

Preferably, said reduction is achieved by an antisense, sense, ribozyme, co-suppression in vivo mutagenesis and/or dominant mutant effect. Therefore, DNA molecules encoding an antisense RNA which is complementary to transcripts of a DNA molecule of the invention are also the subject matter of the present invention, as well as these antisense molecules. Thereby, complementarity does not signify that the encoded RNA has to be 100% complementary. A low degree of complementarity is sufficient, as long as it is high enough in order to inhibit the expression of a protein of the invention upon expression in plant cells. The transcribed RNA is preferably at least 90% and most preferably at least 95% complementary to the transcript of the nucleic acid molecule of the invention. In order to cause an antisense-effect during the transcription in plant cells such DNA molecules have a length of at least 15 bp, preferably a length of more than 100 bp and most preferably a length or more than 500 bp, however, usually less than 5000 bp, preferably shorter than 2500 bp.

The invention further relates to DNA molecules which, during expression in plant cells, lead to the synthesis of an RNA which in the plant cells due to a cosuppression-effect reduces the expression of the nucleic acid molecules of the invention encoding the described protein. The invention also relates to RNA molecules encoded thereby. The principle of the cosuppression as well as the production of corresponding DNA sequences is precisely described, for example, in WO90/12084, Jorgensen, Trends Biotechnol. 8 (1990), 340-344; Niebel et al. (Curr. Top. Microbiol. Immunol. 197 (1995), 91-103; Flavell et al. (Curr. Top. Microbiol. Immunol. 197 (1995), 43-36; Palaqui and Vaucheret, Plant. Mol. Biol. 29 (1995), 149-159; Vaucheret et al., Mol. Gen. Genet. 248 (1995), 311-317; de Borne et al., Mol. Gen. Genet. 243 (1994), 613-621 and in other sources.

Such DNA molecules preferably encode an RNA having a high degree of homology to transcripts of the nucleic acid molecules of the invention. It is, however, not absolutely necessary that the coding RNA is translatable into a protein.

In a further embodiment the present invention relates to DNA molecules encoding an RNA molecule with ribozyme activity which specifically cleaves transcripts of a DNA molecule of the invention as well as these encoded RNA molecules.

Ribozymes are catalytically active RNA molecules capable of cleaving RNA molecules and specific target sequences. By means of recombinant DNA techniques it is possible to alter the specificity of ribozymes. There are various classes of ribozymes. For practical applications aiming at the specific cleavage of the transcript of a certain gene, use is preferably made of representatives of two different groups of ribozymes. The first group is made up of ribozymes which belong to the group I intron ribozyme type. The second group consists of ribozymes which as a characteristic structural feature exhibit the so-called "hammerhead" motif. The specific recognition of the target RNA molecule may be modified by altering the sequences flanking this motif. By base pairing with sequences in the target molecule these sequences determine the position at which the catalytic reaction and therefore the cleavage of the target molecule takes place. Since the sequence requirements for an efficient cleavage are low, it is in principle possible to develop specific ribozymes for practically each desired RNA molecule.

In order to produce DNA molecules encoding a ribozyme which specifically cleaves transcripts of a DNA molecule of the invention, for example a DNA sequence encoding a catalytic domain of a ribozyme is bilaterally linked with DNA sequences which are homologous to sequences encoding the target protein. Sequences encoding the catalytic domain may for example be the catalytic domain of the satellite DNA of the SCMo virus (Davies et al., *Virology* 177 (1990), 216-224 and Steinecke et al., *EMBO J.* 11 (1992), 1525-1530) or that of the satellite DNA of the TobR virus (Haseloff and Gerlach, *Nature* 334 (1988), 585-591). The DNA sequences flanking the catalytic domain are preferably derived from the above-described DNA molecules of the invention. The expression of ribozymes in order to decrease the activity in certain proteins in cells is also known to the person skilled in the art and is, for example, described in EP-A1 0 321 201. The expression of ribozymes in plant cells was, for example, also described, in Feyter et al. (*Mol. Gen. Genet.* 250 (1996), 329-338).

In a preferred embodiment this reduction is effected by means of an antisense effect. For this purpose the DNA molecules of the invention or parts thereof are linked in antisense orientation with a promoter ensuring the transcription in plant cells and possibly with a termination signal ensuring the termination of the transcription as well as the polyadenylation of the transcript. In order to ensure an

efficient antisense effect in the plant cells the synthesized antisense RNA should exhibit a minimum length of 15 nucleotides, preferably of at least 100 nucleotides and most preferably of at least 500 nucleotides. Furthermore, the DNA sequence encoding the antisense RNA should be homologous with respect to the plant species to be transformed. However, DNA sequences exhibiting a high degree of homology to DNA sequences which are present in the cells in endogenic form may also be used, preferably with a homology of more than 95%. To inhibit gene expression of the nucleic acid molecule of the invention, preferably DNA molecules are used that show a homology, i.e. identity to the nucleotide sequences of SEQ ID NO: 1, 7, 9 or 11 of at least 90%, more preferably at least 93%, in particular at least 95% and most preferably at least 98%.

In a further embodiment the reduction of the amount of proteins encoded by the DNA molecules of the invention is effected by a ribozyme effect. The basic effect of ribozymes as well as the construction of DNA molecules encoding such RNA molecules have already been described above. In order to express an RNA with ribozyme activity in transgenic cells the above-described DNA molecules encoding a ribozyme are linked with DNA elements which ensure the transcription in plant cells, particularly with a promoter and a termination signal. The ribozymes synthesized in the plant cells lead to the cleavage of the mRNA encoding the subtilisin-like serine proteases described above.

Furthermore, the subtilisin-like serine protease activity in the plant cells of the invention can also be decreased by the so-called "in vivo mutagenesis" also called "chimeraplasty", for which a hybrid RNA-DNA oligonucleotide ("chimeroplast") is introduced into cells by transformation of cells (Zhu et al., Proc. Natl. Acad. Sci. 96 (1999), 8768-8773, Kipp et al., poster session at the 5<sup>th</sup> International Congress of Plant Molecular Biology, September 21-27, 1997, Singapore; Dixon and Arntzen, meeting report on "Metabolic Engineering in Transgenic Plants", Keystone Symposia, Copper Mountain, CO, USA, TIBTECH 15 (1997), 441-447; WO95/15972; Kren et al., Hepatology 25 (1997), 1462-1468; Cole-Strauss et al., Science 273 (1996), 1386-1389).

Part of the DNA component of the RNA-DNA oligonucleotide is homologous to a nucleic acid sequence of an endogenous subtilisin-like serine protease, in

comparison to the nucleic acid sequence of the endogenous subtilisin-like serine protease it displays, however, a mutation or contains a heterologous region which is surrounded by the homologous regions. By means of base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous nucleic acid molecule followed by a homologous recombination the mutation contained in the DNA component of the RNA-DNA oligonucleotide or the heterologous region can be transferred to the genome of a plant cell. This results in a decrease of the activity.

In addition, the present invention relates to transgenic plants or plant tissue comprising the above described plant cells of the invention.

In a preferred embodiment the transgenic plant displays increased stomatal density, higher conductance of stomata and/or higher content of sugars and protein in plant leaves or other tissue or organs, compared to wild type plants. An increase in the stomatal density is understood to refer to an elevated content of stomata in all aerial plant organs, preferably in the leaves of plants of the present invention in the order of at least about 10% compared to the corresponding non-transformed wild type plant, which already provides for beneficial effects on the vitality of the plant such as, e.g., improved dry matter. Advantageously, the stomatal density is increased by at least about 50%, preferably by more than about 75%, particularly preferred at least about more than 100% and still more preferably more than about 200%. With respect to a decrease in the stomatal density due to the increased expression or activity of subtilisin-like serine proteases according to the invention in the transgenic plant of the invention, the stomatal density is decreased by at least 2%, preferably by more than 5%, particularly preferred at least about more than 10%, and still more preferably more than about 30%. Preferably, the transgenic plant of the invention shows a yield increase, preferably with respect to a harvestable part of the plant.

The term "yield increase" in the present context is understood to mean preferably an increase in production of ingredients, in particular soluble sugars and/or proteins and/or biomass, in particular if measured in fresh or dry weight per plant. An increase in protein and/or sugar content in this context means that the protein content in the plant cells of the invention is increased by at least 5%, preferably by

at least 20%, in particular by at least 50% and most preferably by at least 75% as compared to plant cells of wild type plants that are not modified and/or the sugar content is increased by at least 5%, preferably by at least 25%, in particular by at least 50% and most preferably by at least 75% as compared to plant cells of wild type plants that are not modified.

Methods for determining sugar and protein content are known to the person skilled in the art.

The term "yield increase" means an increase of dry weight by least 3%, preferably by at least 10%, in particular by at least 20% and most preferably by at least 30% and/or an increase in fresh weight by least 2%, preferably by at least 5%, in particular by at least 10% and most preferably by at least 20%.

In yet another aspect, the invention also relates to harvestable parts and to propagation material of the transgenic plants according to the invention which contain transgenic plant cells described above, i.e. at least one recombinant DNA molecule or vector according to the invention and/or which are derived from the above described plants. Harvestable parts can be in principle any useful parts of a plant, for example, leaves, stems, flowers, fruit, seeds, roots etc. Propagation material includes, for example, seeds, fruits, cuttings, seedlings, tubers, rootstocks etc.

In addition, the present invention relates to a kit comprising the recombinant DNA molecule or the vector of the invention. The kit of the invention may contain further ingredients such as selection markers and components for selective media suitable for the generation of transgenic plant cells, plant tissue or plants. The kit of the invention may advantageously be used for carrying out the method of the invention and could be, inter alia, employed in a variety of applications, e.g., in the diagnostic field or as research tool. The parts of the kit of the invention can be packaged individually in vials or in combination in containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art. The kit or its ingredients according to the invention can be used in plant cell and plant tissue culture, for example in agriculture. The kit of the invention and its ingredients are expected to be very

useful in breeding new varieties of, for example, plants which display improved properties such as those described herein.

Thus, the present invention also relates to a method for the production of a transgenic plant comprising an increased yield and/or increased stomatal density compared to wild type plants, wherein

- (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in a decreased activity of a subtilase;
- (b) a plant is regenerated from the cell prepared according to step (a); and
- (c) further plants, if any, are generated from the plant prepared according to step (b).

Likewise, the present invention relates to a method for the production of a transgenic plant having a decreased water consumption and/or decreased stomatal density compared to wild type plants wherein

- (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in an increased activity of a subtilase;
- (b) a plant is regenerated from the cell prepared according to step (a); and
- (c) further plants, if any, are generated from the plant prepared according to step (b).

Furthermore, the present invention relates to use of at least one nucleic acid molecule encoding and/or regulating the expression of a subtilisin-like serine protease, a nucleic acid molecule hybridizing with such a nucleic acid molecule, a nucleic acid molecule encoding a product that interferes with the expression or activity of subtilisin-like serine proteases in plants, or a recombinant DNA molecule or vector of the invention in the production of transgenic plants for increasing yield, and/or increasing stomatal density, and/or increasing leaf fresh and/or dry weight, and/or increasing leaf dry matter content, and/or increasing sugar content in leaves, and/or increasing protein content in leaves, and/or increasing CO<sub>2</sub>-assimilation, and/or sustaining photosynthesis (prevention of photoinhibition) under conditions of high irradiance (see Example 1), and/or changing the water consumption of plants, and or counteracting the consequences



of changing environmental conditions with respect to stomatal density by the inhibition or stimulation of a subtilisin-like serine protease encoding gene. Preferably such nucleic acid molecules are derived from plant genes encoding subtilases. Modulation of the activity of these genes leads to several morphological and physiological changes that are useful for the engineering of improved plants for agriculture, wood culture, or horticulture. Furthermore, the above described nucleic acid molecules and the recombinant DNA molecules and vectors according to the invention may be useful for the alteration or modification of plant/pathogene interaction. The term "pathogen" includes, for example, bacteria, viruses and fungi as well as protozoa. The plants, plant tissue and plant cells of the invention as well as harvestable parts and propagation of such plants can be used for the preparation of feed and food or additives therefor.

#### Deposit

One plasmid produced and used within the scope of the present invention was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) (DSMZ) in Braunschweig, Federal Republic of Germany, which is recognized as an international depository, in accordance with the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. On October 4, 1999 the following plasmid pAH 14/58 was deposited at the German Collection of Microorganisms and Cell Cultures, (DSMZ) (Deposit number): DSM 13076

These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on the Internet, for example under <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Further databases and addresses, such as <http://www.ncbi.nlm.nih.gov/>, <http://www.infobiogen.fr/>, [http://www.fmi.ch/biology/research\\_tools.html](http://www.fmi.ch/biology/research_tools.html), <http://www.tigr.org/>, are known to

the person skilled in the art and can also be obtained using, e.g., <http://www.lycos.com>. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

The Figures show:

**Figure 1:** Top: Release of water vapour (transpiration) from leaves of the wildtype (wt) and the R-558 mutant (R-558) at irradiances of 300  $\mu\text{E}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 1200  $\mu\text{E}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of white light measured by an infrared gas analyzer.

Bottom: Net uptake of  $\text{CO}_2$  (assimilation) into leaves of the wildtype and the R-558 mutant (R-558) at irradiances of 300  $\mu\text{E}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 1200  $\mu\text{E}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of white light measured by an infrared gas analyzer.

**Figure 2:** Schematic representation of the SDD1 protein marked with the amino acid positions bordering the putative pre- and the pro-sequence and the positions of the four invariant amino acids (D, H, N, S) found in all known subtilisins. Furthermore, the consequence of the mutation present in the R-558 mutant is indicated which converts the R codon at amino acid position 492 into a stop codon leading to the formation of a C-terminally truncated protein lacking the essential serine residue at position 552 (S552).

**Figure 3:** Schematic representation of the plasmid pG-SDD1

Fragment A: 7067 bp Sall – EcoRV subfragment of the BAC IGF20D22 that includes the 2328 bp coding region of SDD1 in addition to 2 kb upstream DNA (promoter) and 2.8 kb downstream DNA was inserted into the Sall and SmaI sites of the vector pBIB-Hyg.

Vector: pBIB-Hyg (Becker, 1990, *Nucleic Acids Res.* 18, 203).

**Figure 4:** Schematic representation of the plasmid p35S-SDD1

- Fragment A: 35S promoter of the Cauliflower Mosaic Virus;  
(Gardner et al., 1981, *Nucleic Acids Res.* 9, 2871-2888)
- Fragment B: 2328 nucleotides coding region of the SDD1 gene  
(SEQ ID No. 1)
- Fragment C: polyadenylation signal of the gene 3 from the T-DNA of  
the Ti-plasmid pTi ACH 5 (Gielen et al., 1984, *EMBO J.* 3, 835-846)
- Vector: pBIB-Hyg (Becker, 1990, *Nucleic Acids Res.* 18, 203).

**Figure 5:** Sequence alignments in the four highly conserved domains, the D region, the H region, the substrate binding site, and the S region of the subtilisins Subtilisin BPN' (Wells et al. 1983, *Nucleic Acids Res.* 11, 7911-7925), the KEX2 of yeast (Mizuno et al. 1988, *Biochem. Biophys. Res. Commun.* 156, 246-254), the human FURIN/PACE (Wise et al. 1990, *Proc. Natl. Acad. Sci USA* 87, 9378 - 9382), the human PC1/PC3 (Seidah et al., 1991, *Mol. Endocrinol.* 5, 111 - 122; Smeekens et al., 1991, *Proc. Natl. Acad. Sci. USA* 88, 340 - 344), the CUCUMISIN from *Cucumis melo* (Yamagata et al., 1994, *J. Biol. Chem.* 269, 32725 - 32731), LeP69 from *Lycopersicon esculentum* (Tornero et al., 1996, *Proc. Natl. Acad. Sci. USA* 93, 6332 - 6337), the AG12 from *Alnus glutinosa* (Ribeiro et al., 1995, *Plant Cell* 7, 785 - 794) and of SDD1. The positions of the invariant amino acids are marked with \*. Identical amino acids present at corresponding positions in the different subtilisins are highlighted with black boxes.

**Figure 6:** Schematic representation of the plasmid p35S- $\alpha$ SDD1

- Fragment A: 35S promoter of the Cauliflower Mosaic Virus;  
(Gardner et al., 1981, *Nucleic Acids Res.* 9, 2871-2888)

Fragment B: 2079 bp – fragment (position 74 – 2153 according to the sequence shown in SEQ ID No. 1) of the *SDD1* gene inserted in antisense orientation to the 35S promoter.

Fragment C: polyadenylation signal of the gene 3 from the T-DNA of the Ti-plasmid pTi ACH 5 (Gielen et al., 1984, *EMBO J.* 3, 835-846)

Vector: pBIB-Hyg (Becker, 1990, *Nucleic Acids Res.* 18, 203).

**Figure 7:** Amino acid comparison of three different subtilases; at\_subp1=Subtilase from *Arabidopsis thaliana*, see SEQ ID No. 2; st\_subp1=Subtilase from *Solanum tuberosum*, see SEQ ID No. 8; st\_subp2=Subtilase from *Solanum tuberosum*, see SEQ ID No. 12.

The Examples illustrate the invention:

**Example 1:** H<sub>2</sub>O transpiration and CO<sub>2</sub> assimilation are increased in the *Arabidopsis thaliana* R-558 mutant particularly under conditions of high irradiance

*Arabidopsis thaliana* R-558 mutant plants and corresponding wildtype plants (wt) were grown until bolting in soil (Einheitserde Typ P / Einheitserde Typ T / sand: 2 / 1 / 1) under standard culture conditions in a climatized growth chamber at 16 h photoperiod (180  $\mu\text{mol m}^{-2} \text{s}^{-1}$  fluorescent light; lamp type: TLD36W/840 and TLD36W/830, Philips, Hamburg, Germany) with day and night temperature and relative humidity of 20°C, 60% relative humidity and 16°C, 75% relative humidity, respectively. Single leaves (n=10) were clamped into a gas exchange measurement chamber of an infrared gas analyzer (Walz, Effeltrich, Germany) with H<sub>2</sub>O release from as well as CO<sub>2</sub> uptake into the leaves were measured according to the procedure described by Muschak et al. (*Photosynthetica* 33, 455-465, 1997). As shown in Figure 1, the leaves of the mutant plants showed increased transpiration of H<sub>2</sub>O and increased assimilation (net uptake) of CO<sub>2</sub> under low light (300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and under high light (1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ )

conditions applied during the measurements. Enhancement of CO<sub>2</sub> assimilation, being almost double in the R-558 mutant in comparison to the wild type, was most prominent under the high light conditions which caused a depression of CO<sub>2</sub> assimilation in the wild type in comparison to the low light conditions (photoinhibition).

### Example 2: Isolation of the *SDD1* gene through map-based gene cloning

The genetic locus affected by the mutation in the R-558 mutant has previously been mapped to the top arm of the *Arabidopsis thaliana* chromosome 1 to an interval of approximately 0.59 cM bordered by the molecular markers IGF-20G19LE and IGF-25I3RE (D. Berger, 1997, PhD Thesis Freie Universität Berlin). Two clones of the *Arabidopsis thaliana* genomic IGF-BAC library (Mozo et al., 1998, *Mol. Gen. Genet.* 258, 562-570), IGF20D22 and IGF21M11, which fully cover this region, were sequenced by the SPP consortium (see <http://sequence-www.stanford.edu/ara/SPP.html>) as part of the *Arabidopsis* genome initiative (Bevan et al., 1997, *Plant Cell* 9, 476-478; <http://genome-www.stanford.edu/Arabidopsis/agi.html>). The 0.59 cM region was thus identified to cover 113 kb of genomic DNA sequence. In order to identify the *SDD1* gene corresponding to the mutant locus, this region was scanned for mutations by application of the restriction SSCP- (single strand conformational polymorphism) technique (Dean and Gerrard, 1991, *BioTechniques* 10, 332 - 333; Iwahana et al., 1992, *BioTechniques* 12, 64 - 66) which for this purpose was adapted for the use in plants. This approach is novel and has not been applied for mutation scanning in plants before. Thus, 57 DNA fragments of 2-kb each, were separately PCR amplified from total DNA of wild type and R-558 plants and after digestion with Alu I and/or Hinf I they were analysed through polyacrylamid gel electrophoresis as described by Dean and Gerrard, 1991 (*BioTechniques* 10, 332 - 333) and Iwahana et al., 1992 (*BioTechniques* 12, 64 - 66). A single SSCP was detected that discriminated between the two genotypes and which upon sequencing of the corresponding DNA fragments was shown to be caused by a single C/G -> T/A mutation (Seq. ID No. 1; Seq. ID No. 3). This mutation introduced a premature stop codon into an ORF of a predicted gene spanning 2328 bp that encoded for a

deduced polypeptide of 775 amino acids (Fig. 2; Seq. ID No. 2; Seq. ID No. 4;  
 Genbank                      Accession                      AC002411;                      <http://pgec-genome.pw.usda.gov/F20D22.anno.html#anchor12>).

### **Example 3: Genetic complementation of the R-558 mutant by *Agrobacterium tumefaciens* – mediated DNA-transfer**

In order to confirm the identity of the 2328 bp DNA sequence (Seq. ID No. 1;) as the protein coding region of the *SDD1* gene defective in the R-558 mutant, genetic complementation experiments were performed with the introduction of a wild type DNA-copy into the R-558 mutant through *Agrobacterium tumefaciens* – mediated genetic transformation.

Two plasmids were generated for this purpose:

Plasmid pG-*SDD1*' (Fig. 3) carries the 7067 bp *Sall* - *EcoRV* subfragment of the BAC IGF20D22 that includes the 2328 bp coding region of *SDD1* in addition to 2 kb upstream DNA (promoter) and 2.8 kb downstream DNA was inserted into the *Sall* and *SmaI* sites of the T-DNA vector pBIB-Hyg (Becker, 1990, *Nucleic Acids Res.* 18, 203).

The second plasmid, p35S-*SDD1* (Fig. 4), harbours the three fragments, A, B, C, inserted into the pBIB-Hyg vector (Becker, 1990, *Nucleic Acids Res.* 18, 203). Fragment A, which was inserted between the *EcoRI* and *SacI* restriction sites in the polylinker of pBIB-Hyg, includes the 35S promoter of the Cauliflower Mosaic Virus (CaMV) comprising the nucleotides 7146 through 7464 as described by Gardner et al. (*Nucleic Acids Res.* 9, 2871-2888, 1981). Fragment C contains the polyadenylation signal of the gene 3 from the T-DNA of the Ti-plasmid pTi ACH 5 (Gielen et al., *EMBO J.* 3, 835 – 846, 1984), nucleotides 11749 through 11939 which was isolated as *Pvu* II - *Hind* III fragment from the plasmid pAGV 40 (Herrera-Estrella et al., *Nature* 303, 209 – 213, 1983) and which, after addition of a *Sph* I linker to the *Pvu* II restriction site, was inserted into the *Sph* I and *Hind* III restriction sites of pBIB-Hyg. The resulting intervening plasmid was called pBIN-AR-Hyg. Fragment B covers the 2328 nucleotides coding region of the *SDD1* gene (Seq. ID No. 1) that was amplified by PCR from the BAC IGF20D22 and

provided with Asp718 and XbaI linker sequences and which was inserted into the Asp718 and XbaI restriction sites of pBIN-AR-Hyg.

Both plasmids were separately introduced into *Agrobacterium tumefaciens* according to the procedure described by Höfgen and Willmitzer (*Nucleic Acids Res.* 16, 9877, 1988) and the corresponding T-DNAs were stably introduced into the R-558 mutant by *Agrobacterium tumefaciens* - *in planta* transformation following the method described by Bechtold et al. (*Compt. Rend. Acad. Sci.* 316, 1194 – 1199, 1993). Transformed seedlings selected for antibiotic (Hygromycin) resistance were grown to maturity and tested for the expression of mutant or wildtype phenotypes by microscopic examination of rosette leaves cleared with 80 % ethanol.

**Table 1:** Analysis of stomatal density and distribution in the abaxial epidermices of cotyledons and leaves of wild type (wt), mutant (R-558) and transgenic mutant (R-558 / G-SDD1; R-558 / 35S-SDD1)

Plant	Cotyledon			Primary Leaf			Density [no./mm <sup>2</sup> ]
	Single Stomata <sup>a</sup>	Clustered Stomata <sup>b</sup>	n <sup>c</sup>	Single Stomata <sup>a</sup>	Clustered Stomata <sup>b</sup>	n <sup>c</sup>	
<i>wt</i> #1	100 %	0 %	58	100 %	0 %	163	97.0
<i>wt</i> #2	100 %	0 %	40	100 %	0 %	174	124.3
<i>wt</i> #3	100 %	0 %	51	98.9 %	1.1 %	176	125.7
<i>wt</i> #4	100 %	0 %	49	100 %	0 %	160	114.3
<i>wt</i> #5	100 %	0 %	58	100 %	0 %	166	118.6
<i>R-558</i> #1	61 %	39 %	136	86 %	14 %	492	351.4
<i>R-558</i> #2	56 %	44 %	62	90.1 %	9.9 %	421	300.7
<i>R-558</i> #3	54 %	46 %	137	89.6 %	10.4 %	395	282.1
<i>R-558</i> #4	58 %	42 %	109	93.9 %	6.1 %	409	292.1
<i>R-558</i> #5	60 %	40 %	85	91.6 %	8.4 %	403	287.9
<i>R-558 / G-SDD1</i> #1	100 %	0 %	47	100 %	0 %	279	199.3
<i>R-558 / G-SDD1</i> #2	100 %	0 %	53	83.4 %	16.6 %	181	161.6
<i>R-558 / G-SDD1</i> #3	100 %	0 %	52	100 %	0 %	139	99.3
<i>R-558 / G-SDD1</i> #4	96.4 %	3.6 %	55	100 %	0 %	195	139.3
<i>R-558 / G-SDD1</i> #5	100 %	0 %	53	100 %	0 %	180	128.6
<i>R-558 / G-SDD1</i> #6	100 %	0 %	55	100 %	0 %	169	120.7
<i>R-558 / G-SDD1</i> #7	100 %	0 %	53	100 %	0 %	163	116.4
<i>R-558 / G-SDD1</i> #8	96.8 %	3.2 %	62	100 %	0 %	285	203.6
<i>R-558 / G-SDD1</i> #9	100 %	0 %	37	100 %	0 %	98	70.0
<i>R-558 / G-SDD1</i> #10	96.7 %	3.3 %	60	100 %	0 %	199	142.1
<i>R-558 / 35S-SDD1</i> #1	82 %	18 %	66	100 %	0 %	167	119.3
<i>R-558 / 35S-SDD1</i> #2	100 %	0 %	34	100 %	0 %	123	87.9
<i>R-558 / 35S-SDD1</i> #3	93 %	7 %	55	100 %	0 %	168	120
<i>R-558 / 35S-SDD1</i> #4	74 %	26 %	80	100 %	0 %	136	97.1
<i>R-558 / 35S-SDD1</i> #5	100 %	0 %	45	100 %	0 %	122	87.1
<i>R-558 / 35S-SDD1</i> #6	80 %	20 %	70	100 %	0 %	195	139.3
<i>R-558 / 35S-SDD1</i> #7	53 %	47 %	131	98 %	2 %	200	142.9
<i>R-558 / 35S-SDD1</i> #8	91 %	9 %	68	100 %	0 %	123	87.9
<i>R-558 / 35S-SDD1</i> #9	80 %	20 %	90	95.1 %	4.9 %	123	87.9
<i>R-558 / 35S-SDD1</i> #10	94 %	6 %	65	100 %	0 %	108	77.1

<sup>a</sup> Stomata separated from other stomata by at least one epidermal cell. <sup>b</sup> Stomata placed in direct contact to at least one other stoma. <sup>c</sup> Number of stomata sampled.



As shown in Table 1, 7 out of 10 and 2 out of 10 transformants harbouring the T-DNAs of the pG-*SDD1* or the p35S-*SDD1* plasmids, respectively, showed a wildtype phenotype on cotyledons with respect to the appearance/absence of clustered stomata. 7 transformants carrying the T-DNA of p35S-*SDD1* showed an intermediate phenotype in cotyledons due to inappropriate expression of the transgene in this organ. In primary leaves, all 10 transformants harboring the T-DNA of pG-*SDD1* and all 10 transformants carrying the T-DNA of p35S-*SDD1* showed a strong reduction in stomatal density and/or the fraction of clustered stomata as compared to the R-558 mutant. These data unequivocally demonstrated the identity of the 2325 bp DNA fragment as the coding region of the *SDD1* gene.

#### **Example 4: Analysis of the *SDD1* nucleotide sequence and *SDD1* amino acid sequence**

The analysis of the *SDD1* nucleotide and derived amino acid sequences was performed using the GCG 8.1 and BLAST 2.0 computer programs (see: Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711; Altschul et al. 1997, *Nucleic Acids Res.* 25, 3389-3402).

The derived amino acid sequence of *SDD1* shows significant identity / similarity to known members of a specific class of serine proteases, called subtilisins or dibasic processing endoproteases. In subtilisins, four regions form the catalytic triad and the substrate binding site and are most highly conserved among subtilisins, prominent representatives of which are the bacterial SUBTILISIN BPN' (Wells et al. 1983, *Nucleic Acids Res.* 11, 7911-7925), the KEX2 of yeast (Mizuno et al. 1988, *Biochem. Biophys. Res. Commun.* 156, 246-254), the human furin/PACE (GenBank, Acc. No. X17094) and PC1/PC3 (Seidah et al., 1991, *Mol. Endocrinol.* 5, 111 - 122; Smeekens et al., 1991, *Proc. Natl. Acad. Sci. USA* 88, 340 - 344). Several genes from plants encoding subtilases have been isolated such as CUCUMISIN from *Cucumis melo* (Yamagata et al., 1994, *J. Biol. Chem.* 269, 32725 - 32731), P69 from *Lycopersicon esculentum* (Tornero et al., 1996, *Proc. Natl. Acad. Sci. USA* 93, 6332 - 6337), or AG12 from *Alnus glutinosa*

(Ribeiro et al., 1995, *Plant Cell* 7, 785 - 794). The *in vivo* functions of these plant enzymes, however, are hitherto unknown. In the four conserved regions, SDD1 displays highest sequence similarity to the subtilisins listed above and contains the four characteristic invariant amino acids present in all subtilisins hitherto known (Fig. 5). This unequivocally proves the belonging of SDD1 to this class of endoproteases. The amino acid sequence motif VICAAGNNG within the substrate binding site, however, is unique and distinguishes SDD1 from all other known subtilisins. The mutation present in the R-558 mutant creates a premature stop codon leading to the formation of a C-terminally truncated protein which lacks the essential S-domain containing the catalytically active serine residue (Fig. 2).

#### **Example 5: Modulation of stomatal density in plants through modulation of SDD1 expression by genetic engineering**

The usefulness of the SDD1 gene for the creation of plants with various different levels of increased or decreased stomatal densities through modulation of the degree of SDD1 gene expression was shown by the analysis of further transgenic plants. 4 out of 10 of the transgenic plants carrying the aforementioned (Example 4) T-DNA of p35S-SDD1 and 1 out of 10 of the transgenic plants carrying the aforementioned (Example 4) T-DNA of pG-SDD1 showed lower stomatal density than the corresponding wildtype plants analyzed in parallel (Table 1).

Furthermore, SDD1 antisense inhibition studies were performed. To this end, the plasmid p35S- $\alpha$ SDD1 was generated which contains an antisense-gene construct called ' $\alpha$ SDD1' (Fig. 6). A 2079 bp - fragment (position 74 - 2153 according to the sequence shown in seq. ID 1) of the SDD1 gene was PCR-amplified and subcloned into the pCR 2.1 - vector (Invitrogen, Leek The Netherlands). Using the flanking Asp718 (3') and XbaI (5') restriction sites, the 2 kb SDD1 - fragment was cut from the pCR 2.1 - vector and inserted into the Asp718 and XbaI sites of the pBIN-AR-Hyg. vector (see example 3), thus placing it in antisense orientation to the CaMV 35S - promoter.

The plasmid p35S- $\alpha$ SDD1 was introduced into *Agrobacterium tumefaciens* according to Höfgen and Willmitzer (*Nucleic Acids Res.* 16, 9887, 1988) and was used to generate transgenic *Arabidopsis thaliana* plants through application of the

procedure described by Schmidt and Willmitzer (*Plant Cell Rep.* 7, 583-586, 1988). Among the transgenic plants carrying the T-DNA of p35S- $\alpha$ SDD1 thus generated, individuals with increased stomatal density were obtained.

It was thus demonstrated that through the application of genetic engineering techniques, a gene encoding a subtilisin-like serine protease, can be used to generate plants with various different levels of decreased or increased stomatal densities brought about by the modulation of the expression of said gene.

#### **Example 6: Cloning of two SDD1 homologs from *Solanum tuberosum***

A 2328 bp fragment representing the complete SDD1 coding region from *Arabidopsis* was amplified from the clone IGF20D22 of the *Arabidopsis thaliana* genomic IGF-BAC library (Mozo et al., 1998, *Mol. Gen. Genet.* 258, 562-570) via PCR and was used as a radiolabeled probe (Random Primed DNA Labeling Kit, Boehringer Mannheim) in the screening procedure.

Plaque lifting was performed on  $1.6 \times 10^6$  pfus of a genomic library from *Solanum tuberosum* L. line AM80/5793 (Liu et al., 1991, *Plant Mol. Biol.* 17, 1139-1154), using Hybond N filters (Amersham).

After pre-hybridization for 4 h at 42°C in buffer A (5 x SSC, 0.5 % BSA, 5 x Denhardt, 1 % SDS, 40 mM phosphate buffer, pH 7.2, 100 mg/l herring sperm DNA, 25 % formamid) filters were hybridized to the radiolabeled probe (see above). After 8 h of hybridization the filters were washed 3 times for 20 min at 50°C in a buffer containing 3 x SSC and 0.5 % SDS. X-ray film exposure was usually performed for 14h.

Ten strongly hybridizing phage plaques were rescreened and purified to homogeneity. Phage DNA was prepared according to the method described by Patterson & Dean 1987 (*NAR Vol.15* (15), 6298).

According to their restriction patterns and Southern analysis, using the radiolabeled SDD1 PCR fragment as a probe, phages were divided into two different classes. In case of class I a 3864 bp hybridizing EcoRI/SalI fragment was subcloned into pMCS5 (EcoRI/SalI) (Mo Bi Tec, Göttingen), yielding plasmid pAH10/58. In case of class II a ~4.5 kb hybridizing Scal- fragment was subcloned in pMCS5 (Mo Bi Tec, Göttingen), yielding plasmid pAH 14/58. Both of the plasmids were subjected to DNA-sequencing analysis and contained the

nucleotide sequences, coding for *SDD1* homologs from *Solanum tuberosum* P1gen (SEQ ID NO: 7), P1 - corresponding to P1gen without the intron - (SEQ ID NO: 9), and P2 (SEQ ID NO: 11) The class I a fragments are characterized by the presence of introns, class IIa fragments by the absence of introns. The amino acid sequences encoded by P1 and P2 are shown in SEQ ID NOS: 8, 10 and 12, respectively, and compared to that of *SDD1* in Figure 7.

### Example 7: Overexpression of subtilase in tobacco

A genomic fragment of 2.3 kb was amplified by PCR from the genome of *A. thaliana* var. C24, and Asp 718/ Xba I (the primers comprised these sequences) and cloned into the vector pBinAR Hyg (Höfgen and Willmitzer (1990), Plant Sci. 66: 221-230).

*Agrobacteria* GV2260 (Deblaere (1985), Nucl. Acid Res. 13: 4777-4788) were transformed by way of heat shock, and tobacco plants were infected cv. SNN and regenerated (Rosahl et al., (1987) EMBO J. 6: 1155-1159).

In total, 24 plants were examined as to their RNA expression. For this, RNA from leaf plants was prepared according to Logemann et al. (1987) Anal. Biochem. 163: 16-20) in tissue culture (22°C, 50% atmospheric humidity, 2000 Lux, 16h/8h light rhythm). About 10µg RNA were loaded onto a denaturing gel (Lehrach et al., (1977) Biochem. 16: 4743-4751) and thereupon transferred onto a positively charged membrane Hybond N+ (Amersham Buchler, Braunschweig) via capillary transfer. Thereafter, the RNA was fixed onto the membrane via heat fixation (2h/80°C), washed in 2XSSC for a short period of time (2-3 min), prehybridized for at least 1h at 65°C (0.25M Na-P buffer pH 7.2; 1% BSA, 7% SDS and 10mM EDTA) and hybridized overnight at 65°C with a radioactively labeled probe (Feinberg, and Vogelstein, (1983) Anal. Biochem. 132: 6-13). After two washes with 2XSSC at 65°C for 30 min each the filter was exposed on an X-ray filter overnight at -80°C.

In total, 24 plants were analyzed by way of "Northern technique", of which 12 could be classified as positive, 9 being classified as strong and 3 as weaker expressers. Copies using clear nail varnish were prepared from these plants as well as from the wild type, 5 leafs from different plants of the wild type were analyzed. For doing so, 5 areas each were counted and stomatal density was

determined as stomata/mm<sup>2</sup>. When doing so, it was surprisingly found that stomatal density on the adaxial leaf surface was about twice as high as on the abaxial surface; see table 2.

Table 2

Microscopic analysis of stomatal density  
WT/adaxial

Pl.-Nr						Stomata/m m2
1	71	65	73	68	69	56
2	58	69	62	51	52	47
3	78	74	68	73	76	60
4	83	87	71	82	73	64
5	77	75	79	76	73	62
6	69	69	64	68	63	54
7	61	67	61	64	63	51
MV:						56
STD:						6,13

WT/abaxial

Pl.-Nr.						Stomata/m m2
1	101	108	105	98	109	84
2	128	144	140	139	147	113
3	163	154	160	157	165	129
4	111	115	116	121	107	92
5	183	176	180	168	171	142
6	131	138	135	141	139	111
MV:						112
STD:						21,79

AR  
Subt./adaxial

Pl.Nr.						Stomata/m m2
13	27	21	20	23	21	18
14	17	23	24	22	20	17
15	21	20	23	19	22	17
16	17	13	19	18	23	15
19	24	28	29	26	28	22
22	25	23	27	22	26	20
30	22	26	24	21	25	19
37	27	26	30	33	28	23
38	30	26	33	30	28	24
40	27	23	26	25	31	21
41	21	23	25	22	20	18
42	27	25	22	23	22	19
MV:						19
STD:						2,72

AR  
Subt./abaxial

Pl.Nr.						Stomata/m m2
13	74	71	72	71	76	59
14	73	61	72	70	76	57
15	80	78	71	83	75	63
16	86	99	72	83	97	71
19	84	95	87	93	94	73

22	58	47	62	62	56	46		
30	93	93	96	87	91	75		
37	92	97	95	100	93	77		
38	76	80	78	77	83	64		
40	81	77	88	73	76	64		
41	62	56	61	68	59	50		
42	82	75	78	81	75	63		
mean value					MV:	63	STD.	9,58

	WT	AR
		Subt.
adaxial	56	19
abaxial	112	63

STD = standard deviation  
 MV = mean value  
 WT = wild type  
 AR = transgenic plant

After microscopic analysis of the copies of the transgenic plants which were taken by using clear nail varnish, a decrease in stomatal density by about 50% on both leaf sides could be detected. The hypostomatic phenotype, however, is also found in the transgenic plants; see table 2.

No differences could be found between strong and weak "expressers"; thus even a minor increase of SDD1 activity seems to be sufficient to result in a phenotype.

29-01-2001

PCT/EP99/07633

MAX-PLANCK-GESELLSCHAFT...et al.

Our Ref.: C 1748 PCT

## Claims

1. A recombinant DNA molecule comprising:

- (i) a nucleic acid molecule encoding a subtilisin-like serine protease or encoding a biologically active fragment of such a protein, selected from the group consisting of
  - (a) nucleic acid molecules comprising a nucleotide sequence encoding a protein comprising the amino acid sequence as given in SEQ ID NO: 2, 8, 10 or 12;
  - (b) nucleic acid molecules comprising a nucleotide sequence as given in SEQ ID NO: 1, 7, 9 or 11;
  - (c) nucleic acid molecules encoding a protein comprising at least the D region, H region, substrate binding site and/or S region of the subtilisin-like serine protease encoded by a nucleic acid molecule of (a) or (b); or
  - (d) nucleic acid molecules hybridizing with the complementary strand of a nucleic acid molecule as defined in any one of (a) to (c);
  - (e) nucleic acid molecules encoding a protein the amino acid sequence of which is at least 65% identical to the amino acid sequence encoded by a nucleic acid molecule of any one of (a) to (c);
  - (f) nucleic acid molecules, the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (e); or
- (ii) a nucleic acid molecule encoding a mutant non-active or a hyper-active form of or an antibody against the subtilisin-like serine protease encoded by a nucleic acid molecule of (i); or

AMENDED SHEET

29-01-2001

(iii) a nucleic acid molecule which specifically hybridizes with a nucleic acid molecule of (i) or the complementary strand thereof.

2. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is DNA, cDNA, genomic DNA or synthetically synthesized DNA.
3. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is derived from a plant, preferably Arabidopsis or potato.
4. The recombinant DNA molecule of any one of claims 1 to 3 wherein said nucleic acid molecule is operably linked to regulatory elements allowing the expression of the nucleic acid molecule in plants.
5. A vector comprising a recombinant DNA molecule of any one of claims 1 to 4.
6. A host cell containing a vector of claim 5 or a recombinant DNA molecule of any one of claims 1 to 4.
7. A method for the production of transgenic plants with altered stomata characteristics compared to wild type plants comprising the introduction of a recombinant DNA molecule of any one of claims 1 to 4 or the vector of claim 5 into the genome of a plant, plant cell or plant tissue.
8. A transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5 or obtainable according to the method of claim 7, wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
9. A transgenic plant or a plant tissue comprising plant cells of claim 8.

AMENDED SHEET



29-01-2001

10. The transgenic plant of claim 9 which displays a decreased stomata density, lower conductance of stomata and/or wherein the water consumption is lowered compared to wild type plants.
11. A transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or part thereof, a vector of claim 5 or obtainable according to the method of claim 7, wherein the presence, transcription and/or expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
12. The plant cell of claim 11, wherein the reduction is achieved by an antisense, sense, ribozyme, co-suppression and/or dominant mutant effect.
13. A transgenic plant or plant tissue comprising the plant cells of claim 11 or 12.
14. The transgenic plant of claim 13 which displays increased stomatal density and/or higher conductance of stomata and/or increased content of sugars and/or protein in plant leaves compared to wild type plants.
15. The transgenic plant of any one of claims 9, 10, 13 or 14, the plant cell of any one of claims 8, 11 or 12, or the plant tissue of claim 9 or 13, wherein said plant, plant cell or plant tissue is derived from a monocotyledonous or dicotyledonous plant.
16. The transgenic plant, plant cell or plant tissue of claim 15, wherein said plant is derived from maize, rice, barley, wheat, rye, oats, tomato, melon, banana, chicoree, salad, cabbage, potato, tobacco, alfalfa, clover, oilseed rape, sunflower, peanut, soybean, cotton, sugar beet, linseed, flax, millet, hemp, sugar cane, bean, pea or tree.
17. Harvestable parts or propagation material of plants of any one of claims 9, 10, 13 or 14 to 16 comprising plant cells of claim 8, 11, 12, 15 or 16.

AMENDED SHEET

29-01-2001

18. A kit comprising a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5.
19. A method for the production of a transgenic plant comprising an increased yield and/or increased stomatal density compared to wild type plants, wherein
- (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in a decreased activity of a subtilase;
  - (b) a plant is regenerated from the cell prepared according to step (a); and
  - (c) further plants, if any, are generated from the plant prepared according to step (b).
20. A method for the production of a transgenic plant having a decreased water consumption and/or decreased stomatal density compared to wild type plants wherein
- (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in an increased activity of a subtilase;
  - (b) a plant is regenerated from the cell prepared according to step (a); and
  - (c) further plants, if any, are generated from the plant prepared according to step (b).
21. Use of a nucleic acid molecule encoding or regulating the expression of a subtilisin-like serine protease or a nucleic acid molecule hybridizing with such a nucleic acid molecule, a nucleic acid molecule as defined in any one of claims 1 to 4, a recombinant DNA molecule of any one of claims 1 to 4, or a vector of claim 5 for the production of plants with improved fresh and dry weight, for enhancing the content of sugars and/or protein in plant leaves for the production of plants with reduced leaf temperatures or with reduced water loss and lower water consumption, for the modulation (enhancement) of CO<sub>2</sub> uptake into and H<sub>2</sub>O release from leaves, for sustained photosynthesis under high intensity conditions or for the improvement of disease resistance of plants.

AMENDED SHEET

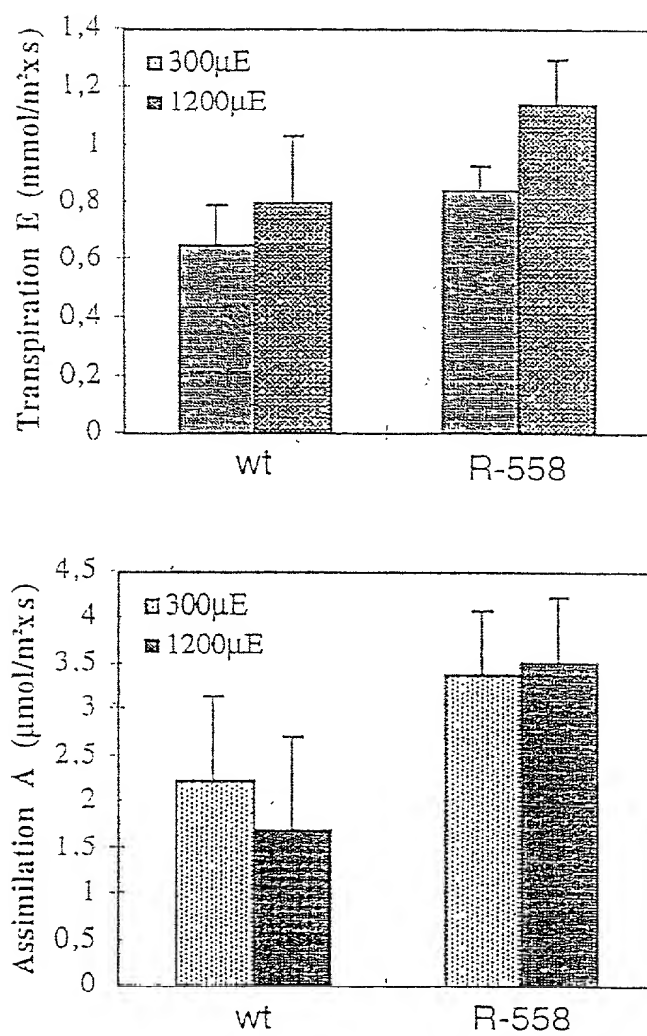


Figure 1

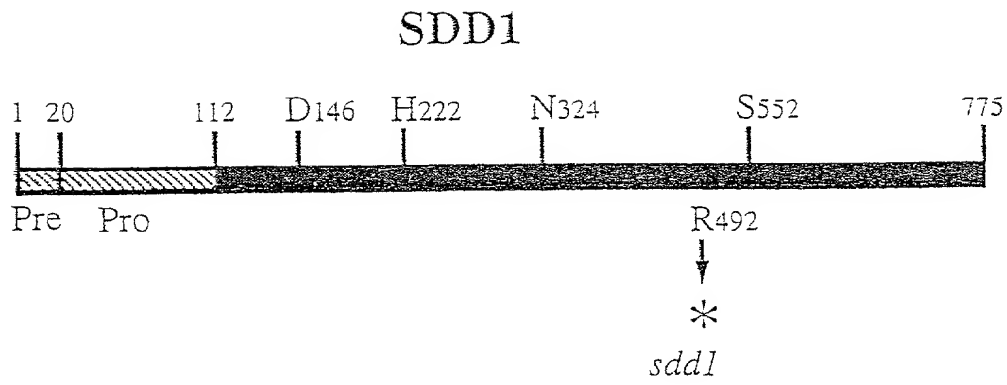


Figure 2

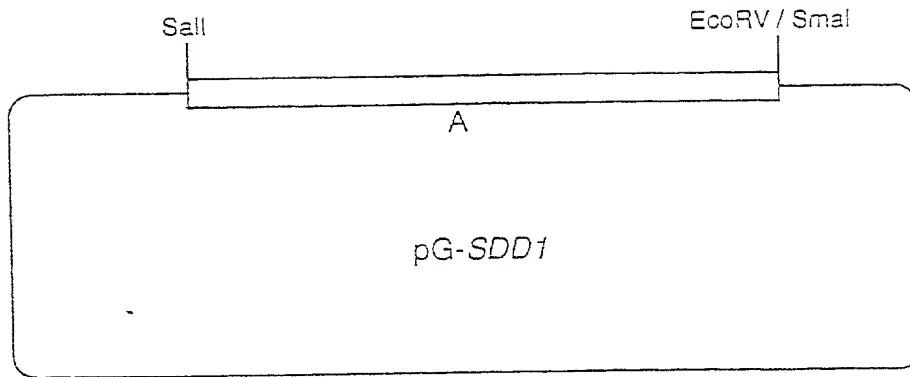


Figure 3

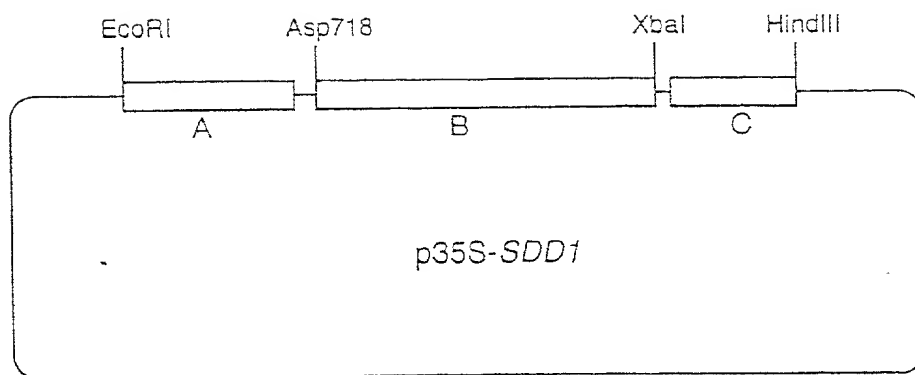


Figure 4

5/9

**D region**

			*
Ag12	136	GEDVIIGVI	DSGVWPESDS FKDDGM
SDD1	137	GQGTIIIGVL	DTGVWPESPS FDDTGM
LeP69	137	GKGVIIIGVI	DTGILPDHPS FSDVGM
Cucumisin	131	ESNIVVGVL	DTGIWPESPS FDDEGF
Furin/PACE	144	GHGIVVSIL	DDGIEKNHPD LAGNYD
PC1/PC3	158	GKGVVITVL	DDGLEWNHTD IYANYD
KEX2	166	GAGVVAIV	DDGLDYENED LKDNFC
Subtilisin BPN'	130	GSNVKVAVI	DSGIDSSHPD LKVAGG
Consensus		G-GV---V-	D-G-----P- --D-G-

**H region**

			*
Furin/PACE	186	YTQMNDNRHG	TRCAGEVAAV ANNGVC
PC1/PC3	200	YDPTNENKHG	TRCAGEIAMQ ANNHKC
KEX2	205	KPRLSDDYHG	TRCAGEIAAK KGNNFC
Cucumisin	196	NGPRDTNGHG	THTASTAAGG LVSQAN
LeP69	195	GSPIDDDGHG	THTASTAAGA FVNGAN
Ag12	208	NSARDTLGHG	THTASTAAGN YVNGAS
SDD1	214	ISARDSTGHG	THTASTVGGG SVSMAN
Subtilisin BPN'	163	NPFQDNNSHG	THVAGTVAAL NNSIGV
Consensus		----D---HG	TH-A-T-A-- --N---

**substrate binding site**

			*
Furin/PACE	284	GLGSIFVWAS	GNGGR
PC1/PC3	298	GKGSIFVWAS	GNGGR
KEX2	303	SKGAIYVFAS	GNGGT
Cucumisin	296	ERGILTSNSA	GNGGP
LeP69	295	ERGILVSCSA	GNNGP
Ag12	307	EKGVVVSTSA	GNAGP
SDD1	313	ERGISVICA	GNNGP
Subtilisin BPN'	251	ASGVVVVAAA	GNEG
Consensus		--G-----AA	GN-G-

**S region**

			*
Furin/PACE	363	SHTGTSAS	APLAAGIIAL TLE
PC1/PC3	377	THTGTSAS	APLAAGIFAL ALE
KEX2	380	SHGGTSAA	APLAAGVYTL LLE
LeP69	527	IISGTSMS	CPHLSGVRAL LKS
SDD1	547	VMSGTSMS	CPHVSGITAL IRS
Ag12	532	MVSGTSMA	CPHASGVAAL LKA
Cucumisin	520	IISGTSMS	CPHITGIATY VKT
Subtilisin BPN'	323	AYNGTSMA	SPHVAGAAAL ILS
Consensus		---GTSMS	-PH--G--AL

Figure 5

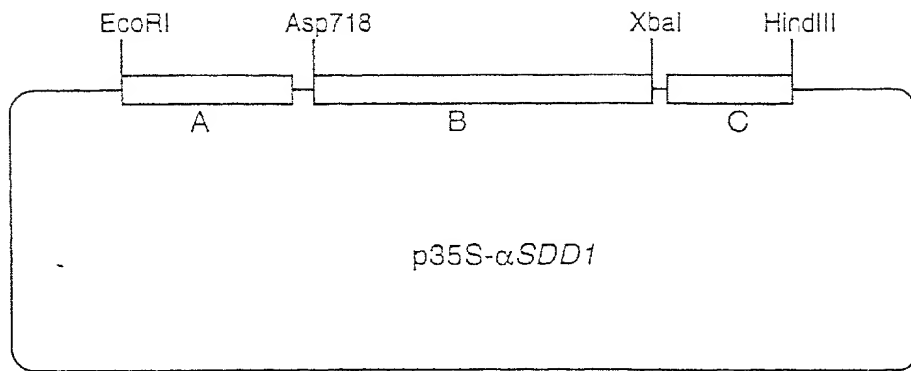


Figure 6



7/9

```

          *           20           *
At_subtila : -----MAPKPFELCIIT : 12
Plgesp     : ILFNPFKYPHQIISTNIPLFNFKYNSMLNFOITYELGCT : 38
St_P2Sca   : -----THLISLGL : 9
                      me F flcf

          40           *           60           *
At_subtila : LIFCSSSSEILQKQTYIVOLHPNSEAKI--FASKEDWH : 49
Plgesp     : LLCEIPLLLQAGNEQTYIVOLHPQHASTRIPFSSKFEQWH : 76
St_P2Sca   : LLCEVC-IGACDEQTYIVOLHPHGATRPP-FSSKLQWH : 45
          LLcf      qaq lQTYIVQLHP at t FsSKfqWH

          80           *           100          *
At_subtila : LSFLQ-----EAVLGVEE : 62
Plgesp     : LSFLENFTNIPLFNFKYIQWNSIPILFLCFYSVYSPAT : 114
St_P2Sca   : LSFLA-----KA--SS : 55
          LSFL                      V V

          120          *           140          *
At_subtila : EE---LEPSSRLLYSYGSATIEGFAAQTHSEATILRYS : 97
Plgesp     : SISSGLENSSRLLYSYHSAFEGFAALLSENELKALKKS : 152
St_P2Sca   : GE---QDSSSRLLYSYHSAMEGFAARLTDEVELLRES : 90
          e e sSRLLYSYhSA EGFAA LtE E e Lr S

          160          *           180          *
At_subtila : PEVAVEDHVLQVQTTYSYKFLGLDGFGNSGVVSKSR : 135
Plgesp     : ANVLESTYELRKLEEVQTTYSYKFLGLSPT-KLGTWKKSE : 189
St_P2Sca   : NDVLESTRAELRLLEIQTTYSYKFLGLSPT-REGAWKKSE : 127
          n Vlsirper LevQTTYSYKFLGLspt eG WlKSg

          200          *           220
At_subtila : FGOCTIIGVLDTGvWPESPSFDDTGMPSTPRKWKGICO : 173
Plgesp     : FGRGAIIGVLDTGvWPESPSFVDHGMSPIPKKWKGXCO : 227
St_P2Sca   : FGRGAIIGVLDTGvWPESPSFDDTGMPPEAPQKWRGVCO : 165
          FGRGAIIGVLDTGvWPESPSFdDhGMppiP KWkG CQ

          *           240          *           260
At_subtila : EGESFSSSSCNRKLI GARFFIRGHRVANSPEESSENMPR : 211
Plgesp     : FGKNFNSSSSCNRKLI GARFFQIGHMMASKISKSIDFM : 265
St_P2Sca   : GGQDFNSSSSCNRKLI GARFFRKGHVASMIS-SPDAV : 202
          eG FnSSSCNRKLI GARFF GHrvAs ts Spd e

          *           280          *           300
At_subtila : EYISARDSTGHGTHFASTVGGSSVSMANVLGNGAGVAR : 249
Plgesp     : DYVSRDSQGHGTHFASTAGGAPVPMASVLGNGAGGAR : 303
St_P2Sca   : EYVSRDSHGHGTHFASTAGGAAPVPMASVLGNGAGGAR : 240
          eYvSprDS GHGTHFASTaGGa VpmA VLGNGAGeAR

```

Figure 7

```

      *           320           *           340
At_subtila : GMAPGAHIAVYKVCWNGCYSSDILAAIDVAIQDKYDV : 287
Plgesp      : GMAPGAHIAVYKVCWSSGCYSSDILAAIDVAIRDGVDI : 341
St_P2Sca    : GMAPGAHIAVYKVCWSSGCYSSDILAAIDVAIRDGVDI : 278
              GMAPGAHIAiYKVCWfsGCYSSDILAAmDVAIRdGVDi

      *           360           *           380
At_subtila : LSLSLGGFPPEPLYDDTIAIGTFRAMERGISVICAAGNN : 325
Plgesp      : LSLSLGGFPVPLYEDTIAIGSFRAMERGISVICAAGNN : 379
St_P2Sca    : LSLSLGGFPPEPLYDDTIAIGSFRAMEHGISVICAAGNN : 316
              LSLSLGGFPiPLYddTIAIGsFRAMErGISVICAAGNN

      *           400           *           4
At_subtila : GPIESSVANTAPWVSTIGAGTLDRRFPAVVRLANGKL : 363
Plgesp      : GPILSSVANEAPWLTATIGASTLDRKFPAIIQLNGNKYV : 417
St_P2Sca    : GPIQSSVANGAPWLTATIGASTLDRRFPA SVQLNGKFK : 354
              GPI SSVAN APWiatIGASTLDRrFPA vqLgNGK l

      20           *           440           *
At_subtila : YGESLYPGKGIKAGREVEVIYVTGDRGSEFCRLRGS : 401
Plgesp      : YGESLYPGKQVHLISQVIELIVYLNIDNGSEFCRLRGS : 455
St_P2Sca    : YGESLYPGKKIPSSQENIELIVYVKIKDRGSEFCRLRGS : 392
              YGESLYPGK v nsqk lEivYv dgDkGSEFCRLRGS

      460           *           480           *
At_subtila : DEELNGKMLICDRGVNGRSEKGEAVKEAGGVAMILAN : 439
Plgesp      : ERAKVHGKILVCDRGVNGRAEKGOVVKESCGVAMILAN : 493
St_P2Sca    : SKAQVRGKMVCDRGVNGRAEKGOVVKESCGVAMILAN : 430
              pra vrGKmVvCDRGVNGRaEKGqvVKEaGGvAMILAN

      500           *           520           *
At_subtila : TEINQEDSDVHLLPATLIGYTESVLLKAYVNATVIRP : 477
Plgesp      : TAVNMEEDSDVHVLPA TLIGEDESTOLOSYMNSTIRKP : 531
St_P2Sca    : TAINMEEDSDVHVLPA TLIGEDESTOLONYNLSTIKRP : 468
              TaiNmEEDSiDVHvLPATLIGfdESiqLq Y NsT kP

      540           *           560           *
At_subtila : KARTIFGGTVIGRSRAPAVAQFSARGPSLANPSTILKPD : 515
Plgesp      : KARTIFGGTVIGKSSAPAVAQFSRGPSFTDPSTILKPD : 569
St_P2Sca    : KARTIFGGTVIGKSRAPAVAQFSRGPSYTDPSILKPD : 506
              tARiIFGGTVIGkSrAPaVAQFSsRGPS tdPSILKPD

      580           *           600
At_subtila : MIAPGVNIIAAMPQNLGPTGLFYDSRRVNFTVMSGTSM : 553
Plgesp      : VIAPGVNIIAAMPQNLGPGSLAFDSRRVNFTVLSGTSM : 607
St_P2Sca    : LIAPGVNIIAAMPQNLGPGSLPDSRRVNFTVMSGTSM : 544
              IAPGVNIIAAMPQNLGPsGLpeDSRRVNFTVmSGTSM

```

Figure 7

```

      *           620           *           640
At_subtila : SCPHVSGITALIRSAAYENNSPAAIKSALMTTADLYDR : 591
Plgesp     : ACPHVSGIAALLHESIAPKWSPAAIKSALMTTADITNEQ : 645
St_P2Sca   : ACPHVSGIAALLHESAPKWTTPAAIRSAALMTTADITADM : 582
             aCPHVSGIaALLhSahPkWsPAAIkSALMTTADt dhq

      *           660           *           680
At_subtila : GKAIKDCNKPAEYFAAGAGHVNPQKAINPGLVYNIQPV : 629
Plgesp     : GKPIWDGDTTRACFAAGAGHVNPGRSDPEGLIYDINAN : 683
St_P2Sca   : GKPIWDGDAPAKIFAAGAGHVNPGRALDPGLIYDIQVD : 620
             GKpImDGd pAglfAiGAGHVNPgraidPGLiYdIq

      *           700           *           720
At_subtila : DYITYLCTLCETFRSDTLAITHKNVSCNGILRNKPGFSL : 667
Plgesp     : DYITHLCTTIGYKNSLILSITHKNVSCHDVLQKNRGFSL : 721
St_P2Sca   : EYITHLCTTIGYRNSLSEVFSITHKNVSCHDILQNNRGFSL : 658
             dYIThLCTiGy nSeilsITHkNVSChdILqkNrGFSL

      *           740           *           760
At_subtila : NYPSIAMTEKRGATTEMITRRVTNVGSPNSIYSVNVKA : 705
Plgesp     : NYPSISVIFKAGKTRKMITRRVTNVGSPNSIYSVEIVA : 759
St_P2Sca   : NYPSISITERRAGMITRIIKRRVTNVGNPNISIYSVDIEA : 696
             NYPSIsviFkaGkTrkmItRRVTNVGSPNSIYSV i A

      *           780           *           8
At_subtila : PEGIKVIIVNPKRLVFKHVDOTLSYRVWFVLKKNRKG : 743
Plgesp     : PEGVKVRVVKPRRLVFKHVNOQLSYRVWFISRKRLCTQ : 796
St_P2Sca   : PEGVKVRVVKPRRLVFKHVNOQLSYRVWFISRKXLES : 733
             PEGvKVRvKPrRLvFKHVnQsLSYRVWFisrK ig k

      00           *           820           *
At_subtila : VASFAQGQLTWVNSHMLMTRVSPISVTLKTN- : 775
Plgesp     : RRSFAEGQLMWINERDKYQKVRSPISVAVWASKK : 829
St_P2Sca   : RMSFAQGQLTWVNVGKATKVKSPISVTLWASML : 766
             r SFAeGQLtW Ns nk qkVrSPISVtwas k

```

Figure 7

Attorney Docket No. 147-223P

**ADDED PAGE TO COMBINED DECLARATION AND POWER OF  
ATTORNEY FOR SIGNING BY ADMINISTRATOR(TRIX),  
EXECUTOR(TRIX) OR LEGAL REPRESENTATIVE ON BEHALF OF  
DECEASED OR INCAPACITATED INVENTOR (37 CFR 1.42 AND 1.43)**

I,

Ms. Janina Lisso*(type or print name(s) of administrator(trix), executor(trix) legal representative or all heirs)*

hereby declare that I am a citizen of

Germanyresiding at Mittelweg 2806862 RoßlauGermanyand that I am executing and signing the declaration to which this is attached as *(check one)*:

the administrator(trix) of



executor(trix) of the last will and testament of



legal representative (or heirs) of

Dieter Berger

Full name of (first, second, etc.) deceased or incapacitated inventor

Germany

Country of citizenship of deceased or incapacitated inventor

Berlin, Germany

Residence (City, State, and Country) of deceased or incapacitated inventor

Detmolderstr. 63, 10715 Berlin, Germany

Mailing Address of deceased or incapacitated inventor

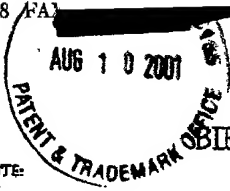
## NOTE:

The name of the first, second, etc. deceased or incapacitated inventor should preferably also be filled in at the appropriate prior space of the declaration adding the words "deceased-completed on added page" or "incapacitated-completed on added page."

That, upon information and belief, I aver those facts which the inventor is required to state.

Date: 9.07.01

[Signature]  
(Signature of administrator(trix), executor(trix)  
legal representative (or all heirs))



DI, US

Attorney Docket No. 147-223P

PLEASE NOTE:  
YOU MUST  
COMPLETE THE  
FOLLOWING

BIRCH, STEWART, KOLASCH &amp; BIRCH, LLP

P.O. Box 747 • Falls Church, Virginia 22040-0747  
Telephone: (703) 705-8000 • Facsimile: (703) 205-8050

## COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title:

MEANS AND METHODS FOR MODULATING STOMATA CHARACTERISTICS IN PLANTS

Fill in Appropriate  
Information -  
For Use Without  
Specification  
Attached:

the specification of which is attached hereto. If not attached hereto,

the specification was filed on April 3, 2001 as

United States Application Number: 09/106,787

and amended on (if applicable) and/or

the specification was filed on October 2, 1999 as PCT

International Application Number PCT/EP 99/0763 and was

amended under PCT Article 19 on (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof more than one year prior to this application; that the same was not in public use or on sale in the United States of America more than one year prior to this application; that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on the invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representative or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority  
Information:  
(if appropriate)

Prior Foreign Application(s)

Priority Claimed

EP 98 119244.6

EPO

October 12, 1998

(Number)

(Country)

(Month/Day/Year Filed)

☐ Yes ☒ No

(Number)

(Country)

(Month/Day/Year Filed)

☐ Yes ☐ No

(Number)

(Country)

(Month/Day/Year Filed)

☐ Yes ☐ No

(Number)

(Country)

(Month/Day/Year Filed)

☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, §119(c) of any United States provisional applications(s) listed below.

Insert Provisional  
Application(s):  
(if any)

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of this Application:

Country

Application Number

Date of Filing (Month/Day/Year)

Insert Requested  
Information:  
(if appropriate)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S.  
Application(s):  
(if any)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

Attorney Docket No. 147-223P

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

Raymond C. Stewart  
Joseph A. Kolasch  
Bernard L. Sweeney  
Charles Gorenstein  
Leonard R. Svensson  
Andrew D. Meikle  
Joe McKinney Mawcy  
John W. Bailey  
Gary D. Yacura  
Mark J. Nuehl

(Reg. No. 21,066)  
(Reg. No. 22,463)  
(Reg. No. 24,448)  
(Reg. No. 29,271)  
(Reg. No. 30,330)  
(Reg. No. 32,868)  
(Reg. No. 32,334)  
(Reg. No. 32,881)  
(Reg. No. 35,416)  
(Reg. No. 36,623)

Tarrell C. Birch  
James M. Stattery  
Michael K. Muter  
Gerald M. Murphy, Jr.  
Terry L. Clark  
Marc S. Weiner  
Donald J. Daley  
John A. Castellano  
Thomas S. Auchterlorie

(Reg. No. 19,382)  
(Reg. No. 28,380)  
(Reg. No. 29,580)  
(Reg. No. 28,772)  
(Reg. No. 32,344)  
(Reg. No. 32,181)  
(Reg. No. 34,113)  
(Reg. No. 35,194)  
(Reg. No. 37,075)

Send Correspondence to:

**BIRCH, STEWART, KOLASCH & BIRCH, LLP**  
P.O. Box 747 • Falls Church, Virginia 22040-0747  
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

or Customer No. 2292

**PLEASE NOTE:  
YOU MUST  
COMPLETE  
THE  
FOLLOWING:**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First  
Inventor (Last, First, Middle Initial)  
Inventor's Signature  
Date of Signature

Residence (City, State & Country)  
Citizenship

Mailing Address (Complete Street Address including City, State & Country)

Full Name of Second  
Inventor, if any  
(Last, First, Middle Initial)

Residence (City, State & Country)  
Citizenship

Mailing Address (Complete Street Address including City, State & Country)

Full Name of Third  
Inventor, if any  
(Last, First, Middle Initial)

Residence (City, State & Country)  
Citizenship

Mailing Address (Complete Street Address including City, State & Country)

Full Name of Fourth  
Inventor, if any  
(Last, First, Middle Initial)

Residence (City, State & Country)  
Citizenship

Mailing Address (Complete Street Address including City, State & Country)

<b>GIVEN NAME/FAMILY NAME</b>	<b>INVENTOR'S SIGNATURE</b>	<b>DATE</b>
BERGER, Dieter	Legal representative: Ms. Janina Liss	
<b>Residence (City, State &amp; Country)</b>	<b>CITIZENSHIP</b>	
Berlin, Germany	German	
<b>MAILING ADDRESS (Complete Street Address including City, State &amp; Country)</b>		
Detmoldstr. 61, 10715 Berlin, Germany		
<b>GIVEN NAME/FAMILY NAME</b>	<b>INVENTOR'S SIGNATURE</b>	<b>DATE</b>
ALTMANN, Thomas		
<b>Residence (City, State &amp; Country)</b>	<b>CITIZENSHIP</b>	
Potsdam, Germany	German	
<b>MAILING ADDRESS (Complete Street Address including City, State &amp; Country)</b>		
Röpkastanenstr. 61, 14469 Potsdam, Germany		
<b>GIVEN NAME/FAMILY NAME</b>	<b>INVENTOR'S SIGNATURE</b>	<b>DATE</b>
<b>Residence (City, State &amp; Country)</b>	<b>CITIZENSHIP</b>	
<b>MAILING ADDRESS (Complete Street Address including City, State &amp; Country)</b>		
<b>GIVEN NAME/FAMILY NAME</b>	<b>INVENTOR'S SIGNATURE</b>	<b>DATE</b>
<b>Residence (City, State &amp; Country)</b>	<b>CITIZENSHIP</b>	
<b>MAILING ADDRESS (Complete Street Address including City, State &amp; Country)</b>		

\*DATE OF SIGNATURE

09/806767

JC08 Rec'd PCT/PTO 03 APR 2001

## SEQUENCE LISTING

&lt;110&gt; Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.

<120> Means and methods for modulating stomata  
characteristica in plants

&lt;130&gt; C1748PCT

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 61

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 2328

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(2325)

&lt;400&gt; 1

atg	gaa	ccc	aaa	cct	ttc	ttt	ctc	tgc	att	atc	ttt	ctt	cta	ttt	tgt	48
Met	Glu	Pro	Lys	Pro	Phe	Phe	Leu	Cys	Ile	Ile	Phe	Leu	Leu	Phe	Cys	
1				5				10					15			

tct	tct	tcg	tca	gag	atc	ctg	cag	aag	cag	act	tac	att	gtt	cag	ctt	96
Ser	Ser	Ser	Ser	Glu	Ile	Leu	Gln	Lys	Gln	Thr	Tyr	Ile	Val	Gln	Leu	
			20				25						30			

cat	cct	aat	agc	gaa	acc	gct	aaa	acc	ttt	gcc	tca	aag	ttt	gat	tgg	144
His	Pro	Asn	Ser	Glu	Thr	Ala	Lys	Thr	Phe	Ala	Ser	Lys	Phe	Asp	Trp	
		35					40					45				

cat	ctt	tct	ttt	ctc	caa	gaa	gcg	gtt	tta	ggc	gtt	gaa	gaa	gaa	gag	192
His	Leu	Ser	Phe	Leu	Gln	Glu	Ala	Val	Leu	Gly	Val	Glu	Glu	Glu	Glu	
	50				55				60							

gaa	gag	cct	tct	tct	cga	ctt	ctc	tac	tcc	tat	ggc	tct	gcg	att	gaa	240
Glu	Glu	Pro	Ser	Ser	Arg	Leu	Leu	Tyr	Ser	Tyr	Gly	Ser	Ala	Ile	Glu	
65					70				75					80		

gga	ttt	gct	gct	cag	ttg	act	gaa	tca	gaa	gcc	gag	ata	ctg	aga	tat	288
Gly	Phe	Ala	Ala	Gln	Leu	Thr	Glu	Ser	Glu	Ala	Glu	Ile	Leu	Arg	Tyr	
			85				90						95			

tca	cct	gaa	gtt	gtt	gca	gtg	aga	cct	gac	cat	gtt	ctt	cag	gtt	caa	336
Ser	Pro	Glu	Val	Val	Ala	Val	Arg	Pro	Asp	His	Val	Leu	Gln	Val	Gln	
		100					105					110				

acc	act	tac	tct	tac	aag	ttc	ttg	gga	ctc	gac	ggc	ttt	gga	aac	tcc	384
Thr	Thr	Tyr	Ser	Tyr	Lys	Phe	Leu	Gly	Leu	Asp	Gly	Phe	Gly	Asn	Ser	
		115				120					125					

ggc	gta	tgg	tct	aaa	tct	cgg	ttt	ggc	caa	ggc	aca	att	atc	ggc	gtg	432
Gly	Val	Trp	Ser	Lys	Ser	Arg	Phe	Gly	Gln	Gly	Thr	Ile	Ile	Gly	Val	
	130					135					140					

ctt	gat	act	gga	gtt	tgg	cct	gaa	agt	cct	agc	ttt	gac	gat	acc	gga	480
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Leu	Asp	Thr	Gly	Val	Trp	Pro	Glu	Ser	Pro	Ser	Phe	Asp	Asp	Thr	Gly		
145					150					155					160		
atg	cct	tcg	att	cca	cgg	aaa	tgg	aaa	ggg	att	tgc	caa	gaa	gga	gaa	528	
Met	Pro	Ser	Ile	Pro	Arg	Lys	Trp	Lys	Gly	Ile	Cys	Gln	Glu	Gly	Glu		
				165				170						175			
agt	ttc	agt	tct	tcg	agc	tgt	aac	cgg	aag	cta	atc	ggc	gct	aga	ttc	576	
Ser	Phe	Ser	Ser	Ser	Ser	Cys	Asn	Arg	Lys	Leu	Ile	Gly	Ala	Arg	Phe		
			180					185					190				
ttc	atc	aga	gga	cac	cgt	gtc	gct	aat	tca	cca	gag	gaa	tca	cca	aac	624	
Phe	Ile	Arg	Gly	His	Arg	Val	Ala	Asn	Ser	Pro	Glu	Glu	Ser	Pro	Asn		
			195					200					205				
atg	cct	cgt	gaa	tac	att	tcc	gca	aga	gat	tca	acg	gga	cac	ggg	act	672	
Met	Pro	Arg	Glu	Tyr	Ile	Ser	Ala	Arg	Asp	Ser	Thr	Gly	His	Gly	Thr		
			210					215				220					
cac	acc	gcc	tca	aca	gtt	ggc	gga	tcc	tct	gtt	tcg	atg	gcg	aat	gtt	720	
His	Thr	Ala	Ser	Thr	Val	Gly	Gly	Ser	Ser	Val	Ser	Met	Ala	Asn	Val		
					225					235					240		
ctt	ggc	aat	gga	gct	ggc	gtg	gct	cgt	ggg	atg	gct	cct	gga	gct	cac	768	
Leu	Gly	Asn	Gly	Ala	Gly	Val	Ala	Arg	Gly	Met	Ala	Pro	Gly	Ala	His		
				245					250					255			
att	gca	gtc	tat	aaa	gtc	tgt	tgg	ttc	aat	ggc	tgt	tac	agc	tct	gac	816	
Ile	Ala	Val	Tyr	Lys	Val	Cys	Trp	Phe	Asn	Gly	Cys	Tyr	Ser	Ser	Asp		
			260					265					270				
att	cta	gca	gct	ata	gat	gta	gcg	att	caa	gat	aaa	gtc	gat	gtt	ctt	864	
Ile	Leu	Ala	Ala	Ile	Asp	Val	Ala	Ile	Gln	Asp	Lys	Val	Asp	Val	Leu		
			275					280					285				
tcg	ctt	tcc	ctt	ggc	ggc	ttc	cct	att	cct	tcg	tat	gat	gac	aca	atc	912	
Ser	Leu	Ser	Leu	Gly	Gly	Phe	Pro	Ile	Pro	Leu	Tyr	Asp	Asp	Thr	Ile		
			290			295					300						
gcc	att	gga	aca	ttc	cga	gcc	atg	gaa	cgc	ggc	ata	tct	gta	atc	tgt	960	
Ala	Ile	Gly	Thr	Phe	Arg	Ala	Met	Glu	Arg	Gly	Ile	Ser	Val	Ile	Cys		
					310					315					320		
gca	gct	ggc	aac	aac	ggc	cca	atc	gaa	agc	tct	gtt	gca	aac	aca	gct	1008	
Ala	Ala	Gly	Asn	Asn	Gly	Pro	Ile	Glu	Ser	Ser	Val	Ala	Asn	Thr	Ala		
				325					330					335			
cct	tgg	gtc	tca	acc	att	ggc	gca	ggc	acg	ctt	gat	cga	aga	ttt	ccc	1056	
Pro	Trp	Val	Ser	Thr	Ile	Gly	Ala	Gly	Thr	Leu	Asp	Arg	Arg	Phe	Pro		
				340					345					350			
gct	gtg	gtc	aga	cta	gcc	aac	gga	aag	ctt	ctc	tat	gga	gag	tca	ttg	1104	
Ala	Val	Val	Arg	Leu	Ala	Asn	Gly	Lys	Leu	Leu	Tyr	Gly	Glu	Ser	Leu		
				355				360					365				
tat	cgc	gga	aaa	ggc	ata	aag	aat	gcc	ggg	aga	gag	gct	gag	gtg	act	1152	
Tyr	Pro	Gly	Lys	Gly	Ile	Lys	Asn	Ala	Gly	Arg	Glu	Val	Glu	Val	Ile		
				370			375				380						
tac	gtc	aca	gga	gga	gat	aaa	gga	agt	gag	ttc	tgt	ttg	aga	ggg	tca	1200	
Tyr	Val	Thr	Gly	Gly	Asp	Lys	Gly	Ser	Glu	Phe	Cys	Leu	Arg	Gly	Ser		
					385		390			395					400		



ctt cca aga gaa gaa atc cga ggc aaa atg gtg att tgt gat cgc gga	1248
Leu Pro Arg Glu Glu Ile Arg Gly Lys Met Val Ile Cys Asp Arg Gly	
405 410 415	
gtc aat gga aga tcg gag aaa gga gaa gcg gtt aaa gaa gct gga gga	1296
Val Asn Gly Arg Ser Glu Lys Gly Glu Ala Val Lys Glu Ala Gly Gly	
420 425 430	
gtt gca atg atc tta gcc aat aca gag atc aac caa gaa gaa gat tct	1344
Val Ala Met Ile Leu Ala Asn Thr Glu Ile Asn Gln Glu Glu Asp Ser	
435 440 445	
att gac gtt cat ctc tta cca gct aca ttg att ggt tac act gag tca	1392
Ile Asp Val His Leu Leu Pro Ala Thr Leu Ile Gly Tyr Thr Glu Ser	
450 455 460	
gtc ctt ctg aag gct tat gtt aat gcc acg gtg aaa cca aag gcg cgg	1440
Val Leu Leu Lys Ala Tyr Val Asn Ala Thr Val Lys Pro Lys Ala Arg	
465 470 475 480	
ata att ttt ggt ggt acg gtg att ggg agg tca cga gca ccg gag gtg	1488
Ile Ile Phe Gly Gly Thr Val Ile Gly Arg Ser Arg Ala Pro Glu Val	
485 490 495	
gct cag ttc tca gct cga gga ccg agt tta gcc aat cct tcg ata cta	1536
Ala Gln Phe Ser Ala Arg Gly Pro Ser Leu Ala Asn Pro Ser Ile Leu	
500 505 510	
aaa ccg gat atg att gct ccg gga gtc aat atc att gcg gct tgg cct	1584
Lys Pro Asp Met Ile Ala Pro Gly Val Asn Ile Ile Ala Ala Trp Pro	
515 520 525	
caa aat cta gga cca acc gga ctt cct tat gat tca aga aga gtt aac	1632
Gln Asn Leu Gly Pro Thr Gly Leu Pro Tyr Asp Ser Arg Arg Val Asn	
530 535 540	
ttc act gta atg tca gga act tca atg tct tgt cca cat gtt agc gga	1680
Phe Thr Val Met Ser Gly Thr Ser Met Ser Cys Pro His Val Ser Gly	
545 550 555 560	
atc act gct ctt atc cgg tct gca tac ccg aac tgg tct cca gct gca	1728
Ile Thr Ala Leu Ile Arg Ser Ala Tyr Pro Asn Trp Ser Pro Ala Ala	
565 570 575	
atc aaa tcc gca ttg atg aca aca gcg gat ttg tac gat cgt caa ggg	1776
Ile Lys Ser Ala Leu Met Thr Thr Ala Asp Leu Tyr Asp Arg Gln Gly	
580 585 590	
aaa gcg ata aag gat ggt aac aaa cca gcc ggt gtg ttt gcg att gga	1824
Lys Ala Ile Lys Asp Gly Asn Lys Pro Ala Gly Val Phe Ala Ile Gly	
595 600 605	
gca ggg cat gtg aat ccg caa aag gcg ata aac ccg gga ttg gtt tac	1872
Ala Gly His Val Asn Pro Gln Lys Ala Ile Asn Pro Gly Leu Val Tyr	
610 615 620	
aac att caa cca gtg gat tac ata act tac ctc tgc act ctt gga ttc	1920
Asn Ile Gln Pro Val Asp Tyr Ile Thr Tyr Leu Cys Thr Leu Gly Phe	
625 630 635 640	
aca aga tca gat att tta gca atc act cat aag aac gtg agc tgc aat	1968
Thr Arg Ser Asp Ile Leu Ala Ile Thr His Lys Asn Val Ser Cys Asn	
645 650 655	

gga ata ttg cgg aaa aac ccg ggt ttt agt ctc aat tac ccg tcg ata 2016  
 Gly Ile Leu Arg Lys Asn Pro Gly Phe Ser Leu Asn Tyr Pro Ser Ile  
 660 665 670

gcc gtg att ttc aaa cgt ggc aag act acg gag atg atc aca agg cgt 2064  
 Ala Val Ile Phe Lys Arg Gly Lys Thr Thr Glu Met Ile Thr Arg Arg  
 675 680 685

gtc act aac gtt ggg agt cct aac tcg ata tac tca gtg aat gtc aag 2112  
 Val Thr Asn Val Gly Ser Pro Asn Ser Ile Tyr Ser Val Asn Val Lys  
 690 695 700

gct cca gag ggg atc aaa gtt att gtc aat cct aag aga ctc gtg ttc 2160  
 Ala Pro Glu Gly Ile Lys Val Ile Val Asn Pro Lys Arg Leu Val Phe  
 705 710 715 720

aaa cac gtg gat cag acg ctg agc tat aga gta tgg ttt gta ttg aag 2208  
 Lys His Val Asp Gln Thr Leu Ser Tyr Arg Val Trp Phe Val Leu Lys  
 725 730 735

aag aaa aac aga gga ggg aag gtg gct agc ttt gca caa ggg cag ttg 2256  
 Lys Lys Asn Arg Gly Gly Lys Val Ala Ser Phe Ala Gln Gly Gln Leu  
 740 745 750

act tgg gtc aac tct cat aat ctg atg cag cga gtt aga agt cca atc 2304  
 Thr Trp Val Asn Ser His Asn Leu Met Gln Arg Val Arg Ser Pro Ile  
 755 760 765

tct gta acc ttg aag act aac tga 2328  
 Ser Val Thr Leu Lys Thr Asn  
 770 775

<210> 2

<211> 775

<212> PRT

<213> Arabidopsis thaliana

<400> 2

Met Glu Pro Lys Pro Phe Phe Leu Cys Ile Ile Phe Leu Leu Phe Cys  
 1 5 10 15

Ser Ser Ser Ser Glu Ile Leu Gln Lys Gln Thr Tyr Ile Val Gln Leu  
 20 25 30

His Pro Asn Ser Glu Thr Ala Lys Thr Phe Ala Ser Lys Phe Asp Trp  
 35 40 45

His Leu Ser Phe Leu Gln Glu Ala Val Leu Gly Val Glu Glu Glu Glu  
 50 55 60

Glu Glu Pro Ser Ser Arg Leu Leu Tyr Ser Tyr Gly Ser Ala Ile Glu  
 65 70 75 80

Gly Phe Ala Ala Gln Leu Thr Glu Ser Glu Ala Glu Ile Leu Arg Tyr  
 85 90 95

Ser Pro Glu Val Val Ala Val Arg Pro Asp His Val Leu Gln Val Gln  
 100 105 110

Thr Thr Tyr Ser Tyr Lys Phe Leu Gly Leu Asp Gly Phe Gly Asn Ser  
 115 120 125

Gly Val Trp Ser Lys Ser Arg Phe Gly Gln Gly Thr Ile Ile Gly Val  
130 135 140

Leu Asp Thr Gly Val Trp Pro Glu Ser Pro Ser Phe Asp Asp Thr Gly  
145 150 155 160

Met Pro Ser Ile Pro Arg Lys Trp Lys Gly Ile Cys Gln Glu Gly Glu  
165 170 175

Ser Phe Ser Ser Ser Ser Cys Asn Arg Lys Leu Ile Gly Ala Arg Phe  
180 185 190

Phe Ile Arg Gly His Arg Val Ala Asn Ser Pro Glu Glu Ser Pro Asn  
195 200 205

Met Pro Arg Glu Tyr Ile Ser Ala Arg Asp Ser Thr Gly His Gly Thr  
210 215 220

His Thr Ala Ser Thr Val Gly Gly Ser Ser Val Ser Met Ala Asn Val  
225 230 235 240

Leu Gly Asn Gly Ala Gly Val Ala Arg Gly Met Ala Pro Gly Ala His  
245 250 255

Ile Ala Val Tyr Lys Val Cys Trp Phe Asn Gly Cys Tyr Ser Ser Asp  
260 265 270

Ile Leu Ala Ala Ile Asp Val Ala Ile Gln Asp Lys Val Asp Val Leu  
275 280 285

Ser Leu Ser Leu Gly Gly Phe Pro Ile Pro Leu Tyr Asp Asp Thr Ile  
290 295 300

Ala Ile Gly Thr Phe Arg Ala Met Glu Arg Gly Ile Ser Val Ile Cys  
305 310 315 320

Ala Ala Gly Asn Asn Gly Pro Ile Glu Ser Ser Val Ala Asn Thr Ala  
325 330 335

Pro Trp Val Ser Thr Ile Gly Ala Gly Thr Leu Asp Arg Arg Phe Pro  
340 345 350

Ala Val Val Arg Leu Ala Asn Gly Lys Leu Leu Tyr Gly Glu Ser Leu  
355 360 365

Tyr Pro Gly Lys Gly Ile Lys Asn Ala Gly Arg Glu Val Glu Val Ile  
370 375 380

Tyr Val Thr Gly Gly Asp Lys Gly Ser Glu Phe Cys Leu Arg Gly Ser  
385 390 395 400

Leu Pro Arg Glu Glu Ile Arg Gly Lys Met Val Ile Cys Asp Arg Gly  
405 410 415

Val Asn Gly Arg Ser Glu Lys Gly Glu Ala Val Lys Glu Ala Gly Gly  
420 425 430

Val Ala Met Ile Leu Ala Asn Thr Glu Ile Asn Gln Glu Glu Asp Ser  
435 440 445

Ile Asp Val His Leu Leu Pro Ala Thr Leu Ile Gly Tyr Thr Glu Ser  
450 455 460

Val Leu Leu Lys Ala Tyr Val Asn Ala Thr Val Lys Pro Lys Ala Arg  
465 470 475 480

Ile Ile Phe Gly Gly Thr Val Ile Gly Arg Ser Arg Ala Pro Glu Val  
485 490 495

Ala Gln Phe Ser Ala Arg Gly Pro Ser Leu Ala Asn Pro Ser Ile Leu  
500 505 510

Lys Pro Asp Met Ile Ala Pro Gly Val Asn Ile Ile Ala Ala Trp Pro  
515 520 525

Gln Asn Leu Gly Pro Thr Gly Leu Pro Tyr Asp Ser Arg Arg Val Asn  
530 535 540

Phe Thr Val Met Ser Gly Thr Ser Met Ser Cys Pro His Val Ser Gly  
545 550 555 560

Ile Thr Ala Leu Ile Arg Ser Ala Tyr Pro Asn Trp Ser Pro Ala Ala  
565 570 575

Ile Lys Ser Ala Leu Met Thr Thr Ala Asp Leu Tyr Asp Arg Gln Gly  
580 585 590

Lys Ala Ile Lys Asp Gly Asn Lys Pro Ala Gly Val Phe Ala Ile Gly  
595 600 605

Ala Gly His Val Asn Pro Gln Lys Ala Ile Asn Pro Gly Leu Val Tyr  
610 615 620

Asn Ile Gln Pro Val Asp Tyr Ile Thr Tyr Leu Cys Thr Leu Gly Phe  
625 630 635 640

Thr Arg Ser Asp Ile Leu Ala Ile Thr His Lys Asn Val Ser Cys Asn  
645 650 655

Gly Ile Leu Arg Lys Asn Pro Gly Phe Ser Leu Asn Tyr Pro Ser Ile  
660 665 670

Ala Val Ile Phe Lys Arg Gly Lys Thr Thr Glu Met Ile Thr Arg Arg  
675 680 685

Val Thr Asn Val Gly Ser Pro Asn Ser Ile Tyr Ser Val Asn Val Lys  
690 695 700

Ala Pro Glu Gly Ile Lys Val Ile Val Asn Pro Lys Arg Leu Val Phe  
705 710 715 720

Lys His Val Asp Gln Thr Leu Ser Tyr Arg Val Trp Phe Val Leu Lys  
725 730 735

Lys Lys Asn Arg Gly Gly Lys Val Ala Ser Phe Ala Gln Gly Gln Leu  
740 745 750

Thr Trp Val Asn Ser His Asn Leu Met Gln Arg Val Arg Ser Pro Ile  
755 760 765

Ser Val Thr Leu Lys Thr Asn  
770 775

<210> 3  
 <211> 2328  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <221> CDS  
 <222> (1)---(1473)

<400> 3  
 atg gaa ccc aaa cct ttc ttt ctc tgc att atc ttt ctt cta ttt tgt 48  
 Met Glu Pro Lys Pro Phe Phe Leu Cys Ile Ile Phe Leu Leu Phe Cys  
 1 5 10 15  
 tct tct tcg tca gag atc ctg cag aag cag act tac att gtt cag ctt 96  
 Ser Ser Ser Ser Glu Ile Leu Gln Lys Gln Thr Tyr Ile Val Gln Leu  
 20 25 30  
 cat cct aat agc gaa acc gct aaa acc ttt gcc tca aag ttt gat tgg 144  
 His Pro Asn Ser Glu Thr Ala Lys Thr Phe Ala Ser Lys Phe Asp Trp  
 35 40 45  
 cat ctt tct ttt ctc caa gaa gcg gtt tta ggt gtt gaa gaa gaa gag 192  
 His Leu Ser Phe Leu Gln Glu Ala Val Leu Gly Val Glu Glu Glu Glu  
 50 55 60  
 gaa gag cct tct tct cga ctt ctc tac tcc tat gcc tct gcg att gaa 240  
 Glu Glu Pro Ser Ser Arg Leu Leu Tyr Ser Tyr Gly Ser Ala Ile Glu  
 65 70 75 80  
 gga ttt gct gct cag ttg act gaa tca gaa gcc gag ata ctg aga tat 288  
 Gly Phe Ala Ala Gln Leu Thr Glu Ser Glu Ala Glu Ile Leu Arg Tyr  
 85 90 95  
 tca cct gaa gtt gtt gca gtg aga cct gac cat gtt ctt cag gtt caa 336  
 Ser Pro Glu Val Val Ala Val Arg Pro Asp His Val Leu Gln Val Gln  
 100 105 110  
 acc act tac tct tac aag ttc ttg gga ctc gac ggt ttt gga aac tcc 384  
 Thr Thr Tyr Ser Tyr Lys Phe Leu Gly Leu Asp Gly Phe Gly Asn Ser  
 115 120 125  
 ggt gta tgg tcc aaa tct cgg ttt ggt caa gcc aca att atc gcc gtg 432  
 Gly Val Trp Ser Lys Ser Arg Phe Gly Gln Gly Thr Ile Ile Gly Val  
 130 135 140  
 ctt gat act gga gtt tgg cct gaa agt cct agc ttt gac gat acc gga 480  
 Leu Asp Thr Gly Val Trp Pro Glu Ser Pro Ser Phe Asp Asp Thr Gly  
 145 150 155 160  
 atg cct tcg att cca cgg aaa tgg aaa ggg att tgc caa gaa gga gaa 528  
 Met Pro Ser Ile Pro Arg Lys Trp Lys Gly Ile Cys Gln Glu Gly Glu  
 165 170 175  
 agt ttc agt tct tcg agc tgt aac cgg aag cta atc ggt gct aga ttc 576  
 Ser Phe Ser Ser Ser Ser Cys Asn Arg Lys Leu Ile Gly Ala Arg Phe  
 180 185 190  
 ttc atc aga gga cac cgt gtc gct aat tca cca gag gaa tca cca aac 624  
 Phe Ile Arg Gly His Arg Val Ala Asn Ser Pro Glu Glu Ser Pro Asn  
 195 200 205  
 atg cct cgt gaa tac att tcc gca aga gat tca acg gga cac ggg act 672

Met	Pro	Arg	Glu	Tyr	Ile	Ser	Ala	Arg	Asp	Ser	Thr	Gly	His	Gly	Thr		
210						215					220						
cac	acc	gcc	tca	aca	gtt	ggg	gga	tcc	tct	gtt	tcg	atg	gcg	aat	gtt	720	
His	Thr	Ala	Ser	Thr	Val	Gly	Gly	Ser	Ser	Val	Ser	Met	Ala	Asn	Val		
225					230					235					240		
ctt	ggc	aat	gga	gct	ggg	gtg	gct	cgt	ggg	atg	gct	cct	gga	gct	cac	768	
Leu	Gly	Asn	Gly	Ala	Gly	Val	Ala	Arg	Gly	Met	Ala	Pro	Gly	Ala	His		
				245					250					255			
att	gca	gtc	tat	aaa	gtc	tgt	tgg	ttc	aat	ggg	tgt	tac	agc	tct	gac	816	
Ile	Ala	Val	Tyr	Lys	Val	Cys	Trp	Phe	Asn	Gly	Cys	Tyr	Ser	Ser	Asp		
			260					265					270				
att	cta	gca	gct	ata	gat	gta	gcg	att	caa	gat	aaa	gtc	gat	gtt	ctt	864	
Ile	Leu	Ala	Ala	Ile	Asp	Val	Ala	Ile	Gln	Asp	Lys	Val	Asp	Val	Leu		
		275					280					285					
tgc	ctt	tcc	ctt	ggc	ggg	ttc	cct	att	cct	ttg	tac	gat	gac	aca	atc	912	
Ser	Leu	Ser	Leu	Gly	Gly	Phe	Pro	Ile	Pro	Leu	Tyr	Asp	Asp	Thr	Ile		
	290					295					300						
gcc	att	gga	aca	ttc	cga	gcc	atg	gaa	cgc	ggg	ata	tct	gta	atc	tgt	960	
Ala	Ile	Gly	Thr	Phe	Arg	Ala	Met	Glu	Arg	Gly	Ile	Ser	Val	Ile	Cys		
305					310					315					320		
gca	gct	ggg	aac	aac	ggg	cca	atc	gaa	agc	tct	gtt	gca	aac	aca	gct	1008	
Ala	Ala	Gly	Asn	Asn	Gly	Pro	Ile	Glu	Ser	Ser	Val	Ala	Asn	Thr	Ala		
				325					330					335			
cct	tgg	gtc	tca	acc	att	ggc	gca	ggc	acg	ctt	gat	cga	aga	ttt	ccc	1056	
Pro	Trp	Val	Ser	Thr	Ile	Gly	Ala	Gly	Thr	Leu	Asp	Arg	Arg	Phe	Pro		
			340					345					350				
gct	gtg	gtc	aga	tta	gcc	aac	gga	aag	ctt	ctc	tat	gga	gag	tca	ttg	1104	
Ala	Val	Val	Arg	Leu	Ala	Asn	Gly	Lys	Leu	Leu	Tyr	Gly	Glu	Ser	Leu		
		355					360				365						
tat	ccg	gga	aaa	ggg	ata	aag	aat	gcc	ggg	aga	gag	gtt	gag	gtg	att	1152	
Tyr	Pro	Gly	Lys	Gly	Ile	Lys	Asn	Ala	Gly	Arg	Glu	Val	Glu	Val	Ile		
	370					375					380						
tac	gtc	aca	gga	gga	gat	aaa	gga	agt	gag	ttc	tgt	ttg	aga	ggg	tca	1200	
Tyr	Val	Thr	Gly	Gly	Asp	Lys	Gly	Ser	Glu	Phe	Cys	Leu	Arg	Gly	Ser		
385					390					395					400		
ctt	cca	aga	gaa	gaa	atc	cga	ggc	aaa	atg	gtg	att	tgt	gat	cgc	gga	1248	
Leu	Pro	Arg	Glu	Glu	Ile	Arg	Gly	Lys	Met	Val	Ile	Cys	Asp	Arg	Gly		
				405					410					415			
gtc	aat	gga	aga	tgc	gag	aaa	gga	gaa	gcg	gtt	aaa	gaa	gct	gga	gga	1296	
Val	Asn	Gly	Arg	Ser	Glu	Lys	Gly	Glu	Ala	Val	Lys	Glu	Ala	Gly	Gly		
		420						425				430					
gtt	gca	atg	atc	tta	gcc	aat	aca	gag	atc	aac	caa	gaa	gaa	gat	tct	1344	
Val	Ala	Met	Ile	Leu	Ala	Asn	Thr	Glu	Ile	Asn	Gln	Glu	Glu	Asp	Ser		
		435					440					445					
att	gac	gtt	cat	ctc	tta	cca	gct	aca	ttg	att	ggg	tac	act	gag	tca	1392	
Ile	Asp	Val	His	Leu	Leu	Pro	Ala	Thr	Leu	Ile	Gly	Tyr	Thr	Glu	Ser		
	450					455					460						

gtc ctt ctg aag gct tat gtt aat gcc acg gtg aaa cca aag gcg cgg 1440  
 Val Leu Leu Lys Ala Tyr Val Asn Ala Thr Val Lys Pro Lys Ala Arg  
 465 470 475 480

ata att ttt ggt ggt acg gtg att ggg agg tca tgagcaaccg aggtgggtca 1493  
 Ile Ile Phe Gly Gly Thr Val Ile Gly Arg Ser  
 485 490

gttttcagct cgaggaccga gtttagccaa tccttcgata ctaaaaccgg atatgattgc 1553

tccgggagtc aatatcattg cggcttggcc tcaaaatcta ggaccaaccg gacttcctta 1613

tgattcaaga agagttaact tcaactgtaat gtcaggaact tcaatgtctt gtccacatgt 1673

tagcgggaatc actgctctta tccgggtctgc ataccogaac tgggtctccag ctgcaatcaa 1733

atccgcattg atgacaacag cggatttgta cgatcgtaaa gggaaagcga taaaggatgg 1793

taacaaacca gccgggtgtgt ttgcgattgg agcagggcat gtgaatccgc aaaaggcgat 1853

aaaccoggga ttggtttaca acattcaacc agtggattac ataacttacc tctgcactct 1913

tggattcaca agatcagata ttttagcaat cactcataag aacgtgagct gcaatggaat 1973

attgcggaaa aaaccogggtt ttagtctcaa ttaccgctcg atagccgtga ttttcaaacy 2033

tggcaagact acggagatga tcacaaggcg tgtcactaac gttgggagtc ctaactcgat 2093

ataactcagtg aatgtcaagg ctccagaggg gatcaaagtt attgtcaatc ctaagagact 2153

tgtgttcaaa cacgtggatc agacgtgag ctatagagta tgggttgtat tgaagaagaa 2213

aaacagagga gggaagggtgg ctagctttgc acaagggcag ttgacttggg tcaactctca 2273

taatctgatg cagcgagtta gaagtccaat ctctgtaacc ttgaagacta actga 2328

<210> 4

<211> 491

<212> PRT

<213> Arabidopsis thaliana

<400> 4

Met Glu Pro Lys Pro Phe Phe Leu Cys Ile Ile Phe Leu Leu Phe Cys  
 1 5 10 15

Ser Ser Ser Ser Glu Ile Leu Gln Lys Gln Thr Tyr Ile Val Gln Leu  
 20 25 30

His Pro Asn Ser Glu Thr Ala Lys Thr Phe Ala Ser Lys Phe Asp Trp  
 35 40 45

His Leu Ser Phe Leu Gln Glu Ala Val Leu Gly Val Glu Glu Glu Glu  
 50 55 60

Glu Glu Pro Ser Ser Arg Leu Leu Tyr Ser Tyr Gly Ser Ala Ile Glu  
 65 70 75 80

Gly Phe Ala Ala Gln Leu Thr Glu Ser Glu Ala Glu Ile Leu Arg Tyr  
 85 90 95

Ser Pro Glu Val Val Ala Val Arg Pro Asp His Val Leu Gln Val Gln  
 100 105 110

Thr Thr Tyr Ser Tyr Lys Phe Leu Gly Leu Asp Gly Phe Gly Asn Ser  
 115 120 125  
 Gly Val Trp Ser Lys Ser Arg Phe Gly Gln Gly Thr Ile Ile Gly Val  
 130 135 140  
 Leu Asp Thr Gly Val Trp Pro Glu Ser Pro Ser Phe Asp Asp Thr Gly  
 145 150 155 160  
 Met Pro Ser Ile Pro Arg Lys Trp Lys Gly Ile Cys Gln Glu Gly Glu  
 165 170 175  
 Ser Phe Ser Ser Ser Ser Cys Asn Arg Lys Leu Ile Gly Ala Arg Phe  
 180 185 190  
 Phe Ile Arg Gly His Arg Val Ala Asn Ser Pro Glu Glu Ser Pro Asn  
 195 200 205  
 Met Pro Arg Glu Tyr Ile Ser Ala Arg Asp Ser Thr Gly His Gly Thr  
 210 215 220  
 His Thr Ala Ser Thr Val Gly Gly Ser Ser Val Ser Met Ala Asn Val  
 225 230 235 240  
 Leu Gly Asn Gly Ala Gly Val Ala Arg Gly Met Ala Pro Gly Ala His  
 245 250 255  
 Ile Ala Val Tyr Lys Val Cys Trp Phe Asn Gly Cys Tyr Ser Ser Asp  
 260 265 270  
 Ile Leu Ala Ala Ile Asp Val Ala Ile Gln Asp Lys Val Asp Val Leu  
 275 280 285  
 Ser Leu Ser Leu Gly Gly Phe Pro Ile Pro Leu Tyr Asp Asp Thr Ile  
 290 295 300  
 Ala Ile Gly Thr Phe Arg Ala Met Glu Arg Gly Ile Ser Val Ile Cys  
 305 310 315 320  
 Ala Ala Gly Asn Asn Gly Pro Ile Glu Ser Ser Val Ala Asn Thr Ala  
 325 330 335  
 Pro Trp Val Ser Thr Ile Gly Ala Gly Thr Leu Asp Arg Arg Phe Pro  
 340 345 350  
 Ala Val Val Arg Leu Ala Asn Gly Lys Leu Leu Tyr Gly Glu Ser Leu  
 355 360 365  
 Tyr Pro Gly Lys Gly Ile Lys Asn Ala Gly Arg Glu Val Glu Val Ile  
 370 375 380  
 Tyr Val Thr Gly Gly Asp Lys Gly Ser Glu Phe Cys Leu Arg Gly Ser  
 385 390 395 400  
 Leu Pro Arg Glu Glu Ile Arg Gly Lys Met Val Ile Cys Asp Arg Gly  
 405 410 415  
 Val Asn Gly Arg Ser Glu Lys Gly Glu Ala Val Lys Glu Ala Gly Gly  
 420 425 430  
 Val Ala Met Ile Leu Ala Asn Thr Glu Ile Asn Gln Glu Glu Asp Ser  
 435 440 445



Ile Asp Val His Leu Leu Pro Ala Thr Leu Ile Gly Tyr Thr Glu Ser  
450 455 460

Val Leu Leu Lys Ala Tyr Val Asn Ala Thr Val Lys Pro Lys Ala Arg  
465 470 475 480

Ile Ile Phe Gly Gly Thr Val Ile Gly Arg Ser -  
485 490

<210> 5

<211> 1970

<212> DNA

<213> Arabidopsis thaliana

<400> 5

```
gtgactttg attcaagctt tgtttcgatt gattgagcca actgctggaa aaattactat 60
tgacaacatt gacatttctc aaattgggtc tcatgatctt cgtagtccgc ttgggattat 120
acctcaagat cctacattat ttgaaggaac aatccgagca aatcttgacc cacttgaaga 180
acattcagat gataaaatct gggaggatc tataaatatg ttgtttgata ctcttatctt 240
gtttatgttt tagacactaa actctgagat ttggagtttg attctagaga tttacccaac 300
tttgcgtgaca ggcgcttgat aaatcccagc ttggagacgt tgttagagga aaagacctaa 360
aaacttgactc tccaggtaac tatttacatc aaaagtcctc tctttttccc gggctctttt 420
cgcttctttc tactgatttt tttggctcaa aaccgagtag tactggaaaa tggagataac 480
tggagtgtag ggcagagaca gcttgtgtca cttggacgag cactactgaa acaagccaaa 540
atacttgttc ttgatgaagc aacagcatcg gttgacacag caacagacaa tctgatccag 600
aagataatca gaacagagtt tgaagactgc acggtctgca ccattgctca ccggatacca 660
actgtttatg acagtgcctt agttttgggt ctcagcgacg gttagtctca taaaaattaa 720
aaacatggat ctttcttcat attactcgtc gtcttttggg agaattcaat gtttatgttt 780
atgggtttgtt gcaggttagg tagcagagtt tgatactcct gcacggctat tagaagacaa 840
atcatcgatg ttcttgaaat tggtaacaga atactcctca agatctactg gaatccctga 900
attatgatcc tccatgttaa aaattcagtt taggggggtt ctttctcaa gaggatataa 960
aagaactgat atgtgacaaa agcttaaggt ctaaagtaag agagagtttt ccacagggtt 1020
taagaaaaga aaaagcatga aaggatgcca aaatctccgc gcttaaaaaa ctttgggttt 1080
aaatctcttc tgtcgaacat tgggagaaac tttttttgta tggaaacagt agtttctttg 1140
gttttctgtt ttatcaataa actgcaaaaa caaacaaga ttagagtaga aactactaat 1200
cattogtcat catccctcaa gtgtgatctg tattgggctt attataagtg taatcagtag 1260
tgggctttata agttgtacag agcccaatat aacaatatct gtacgacgtc gttttgttgt 1320
gacgtagtac ccgatgacaa aacgaggcgt gttgagcgtg cgtgtataaa tgcacgtagt 1380
gaacgaacac taaccacgct gctgcattta attcctttct caacggctgt attttcctat 1440
tcaaggcttt aactaagttt aatagtatgt ttttaaaaaa atacatatat ttggccaatg 1500
gttgattatt aagttattgt taaatgaatt ttctttggct tggtaaaact tcaatggaaa 1560
ccaaataaat ttcaaatctt tttgttgcgt aaaaaggcta caaaaactac tatacgcaat 1620
aaaacctaac cataacatct cgtgaacaag gaaaaaaaaa gaaaaaacag agacacagag 1680
acaaggcaga caaaaaagcc ttacaagtga aaaaccttaa acagcttcag ttaatatagt 1740
tcatgtctta gaaaaaaata aaagagaaga agctcctacc tctgcaacat acaaaggata 1800
ctcgtgagggc agagctctta ctcttcacga gcttcacat cttcttcttt agactccaaa 1860
tcccagggtt tgaattctct tcattcttct ttaaatacco tattcttctt cttctctaaa 1920
accatgcact tcaactacac taaaccaact ctttttttta actctctcca 1970
```

<210> 6

<211> 1140

<212> DNA

<213> Arabidopsis thaliana

<400> 6

```
aaatcatcga tgttcttgaa attggtaaca gaatactcct caagatctac tggaaatccct 60
gaattatgat cctccatggt aaaaattcag tttaggggtt ttcttttctc aagaggatat 120
aaaagaactg atatgtgaca aaagcttaag gtctaaagta agagagagtt ttccacaggg 180
tttaagaaaa gaaaaagcat gaaaggatgc caaatctcc gcgcttaaaa aactttgggt 240
```

```

ttaaattctct tctgtcgaac attgggagaa accctctcttg tatggaacag ttagtttctt 300
tggttttcat gtttatcaat aaactgcaaa aacaaacaaa gattagagta gaaactacta 360
atcattcgtc atcatccctc aagtgatgc tgtattgggc ttattataag tgtaatcagt 420
actgggctta taagttgtac agagcccaat ataacaatat ctgtacgacg tcgttttgtt 480
gtgacgtagt acccgatgac aaaacgaggc gtgttgagcg tgcgtgtata aatgcaogta 540
gtgaacgaac actaaccacg ctgctgcatt taattccttt ctcaacggtc gtattttctt 600
attcaaggct ttaactaagt ttaatagtat gtttttaaaa aaatacatat atttggtcaa 660
tggttgatta ttaagttatt gttaaataaa tttctcttgg cttggtaaaa cttcaatgga 720
aaccaaataa atttcaaaat cttttgttgc tgaanaaggc tacacaaact actatacgca 780
ataaaaacta accataacat ctctgaaca aggaaaaaaa aaggaaaaac agagacacag 840
agacaaggca gacaaaaaag cttacaagt gaaaaacctt aaacagcttc agttaatata 900
gttcattgtc tagaaaaaaa taaaagagaa gaagctccta cctctgcaac acacaaagga 960
tactcgtgag gcagagctct tactcttcac gagcttcata atcttcttcc ttagactcca 1020
aatcccaggc tttgaattct ctccattctt ctttaaatac cctattcttc ttcttctcta 1080
aaaccatgca cttcaactac actaaaccaa ctctcttttt taactctccc caatggaacc 1140

```

```

<210> 7
<211> 3865
<212> DNA
<213> Solanum tuberosum

```

```

<220>
<221> exon
<222> (3)..(551)

```

```

<220>
<221> intron
<222> (552)..(966)

```

```

<220>
<221> exon
<222> (967)..(1654)

```

```

<220>
<221> intron
<222> (1655)..(1737)

```

```

<220>
<221> exon
<222> (1738)..(2222)

```

```

<220>
<221> intron
<222> (2223)..(2485)

```

```

<220>
<221> exon
<222> (2486)..(3252)

```

```

<220>
<221> CDS
<222> (3)..(551)

```

```

<220>
<221> CDS
<222> (967)..(1653)

```

```

<220>
<221> CDS
<222> (1737)..(2222)

```

```

<220>

```

&lt;221&gt; CDS

&lt;222&gt; (2486)..(3253)

&lt;400&gt; 7

```

ga att ctg ttc aac ccc ttt aaa tac ccc cat caa att ata tca aca 47
Ile Leu Phe Asn Pro Phe Lys Tyr Pro His Gln Ile Ile Ser Thr
1 5 10 15

aac att cca tta ttc aac ttc aaa tat aat tca atg gaa ctc aat ttc 95
Asn Ile Pro Leu Phe Asn Phe Lys Tyr Asn Ser Met Glu Leu Asn Phe
20 25 30

caa ttc tac ttt ctc tgt ttt cta ctc tgt ttt att ccc ctg cta caa 143
Gln Phe Tyr Phe Leu Cys Phe Leu Cys Phe Ile Pro Leu Leu Gln
35 40 45

gct caa aat ttg caa act tat ata gta caa tta cat cca caa cat gca 191
Ala Gln Asn Leu Gln Thr Tyr Ile Val Gln Leu His Pro Gln His Ala
50 55 60

tca aca aga acc cct ttt agt tct aaa ttt cag tgg cac ctt tca ttt 239
Ser Thr Arg Thr Pro Phe Ser Ser Lys Phe Gln Trp His Leu Ser Phe
65 70 75

ctt gaa aat ttc aca aac att cca tta ttc aac ttc aaa tat att caa 287
Leu Glu Asn Phe Thr Asn Ile Pro Leu Phe Asn Phe Lys Tyr Ile Gln
80 85 90 95

tgg aac tca att cca att cta ttt ctc tgt ttc tac tct gtt tat tcc 335
Trp Asn Ser Ile Pro Ile Leu Phe Leu Cys Phe Tyr Ser Val Tyr Ser
100 105 110

cct gct aca agc att tcc tca ggt gaa aac tcg agt tct cgc ctt ttg 383
Pro Ala Thr Ser Ile Ser Ser Gly Glu Asn Ser Ser Ser Arg Leu Leu
115 120 125

tac tct tac cat tct gca ttt gaa ggt ttt gca gca ctt cta tct gaa 431
Tyr Ser Tyr His Ser Ala Phe Glu Gly Phe Ala Ala Leu Leu Ser Glu
130 135 140

aat gag cta aag gca ctg aag aaa tcg aat aat gtg tta tca ata tat 479
Asn Glu Leu Lys Ala Leu Lys Lys Ser Asn Asn Val Leu Ser Ile Tyr
145 150 155

ccg gag agg aag ctt gag gtt caa aca act tat tct tac aag ttc tta 527
Pro Glu Arg Lys Leu Glu Val Gln Thr Thr Tyr Ser Tyr Lys Phe Leu
160 165 170 175

gga ctt agt cct aca aag gaa ggt atgttacata gtttgaata tatataaagt 581
Gly Leu Ser Pro Thr Lys Glu Gly
180

tgaggaaaca atgagtcata aggctttatt tattaatatga ctagtctgga cttagagtgtc 641
acacgttagga ttttaaacttg tgaattaagg aaaattctta aacccctttt acaactgttc 701
tggagtcatt tcttatgtca atgtgattca acagttcata tataacaaaa aggatttgtc 761
tttaccttgt ttccgaagta aaaatatttc aacgaagggg attcaaccga accccatttg 821
ttcccttcaa ctctgctaag agatattttc tatgtatttc atttaagact tgaattttta 881
agcttttaaat ttcttatgtt cctctggagc ctttctctctn acttactttt atactgtctt 941

```

tgtatctttt tttctctaataaaggt	act tgg tca aag tct gga ttt ggt cga	993
	Thr Trp Leu Lys Ser Gly Phe Gly Arg	
	185 190	
ggc gcg atc att gga gtt ctt gat act gga att tgg cca gaa agt cca	1041	
Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Ile Trp Pro Glu Ser Pro		
195 200 205		
agt ttt gtt gat cat gga atg tct cct att cca aag aaa tgg aaa ggt	1089	
Ser Phe Val Asp His Gly Met Ser Pro Ile Pro Lys Lys Trp Lys Gly		
210 215 220		
ntc tgc caa gaa gga aaa aac ttc aat tct tca agt tgc aat cgc aag	1137	
Xaa Cys Gln Glu Gly Lys Asn Phe Asn Ser Ser Ser Cys Asn Arg Lys		
225 230 235 240		
ctt att ggt gca agg ttt ttc cag ata gga cac atg atg gca tca aag	1185	
Leu Ile Gly Ala Arg Phe Phe Gln Ile Gly His Met Met Ala Ser Lys		
245 250 255		
aca tca aaa tca ata gat ttt atg gag gat tat gta tca cct cga gat	1233	
Thr Ser Lys Ser Ile Asp Phe Met Glu Asp Tyr Val Ser Pro Arg Asp		
260 265 270		
tct caa ggc cat ggt aca cat aca gca tct act gca ggg gga gct ccc	1281	
Ser Gln Gly His Gly Thr His Thr Ala Ser Thr Ala Gly Gly Ala Pro		
275 280 285		
gtt cca atg gcg agt gtg ctt gga aat gga gca gga gag gct cga ggg	1329	
Val Pro Met Ala Ser Val Leu Gly Asn Gly Ala Gly Glu Ala Arg Gly		
290 295 300		
atg gcc cct ggt gct cat atc gcg ata tac aaa gtt tgt tgg tct agt	1377	
Met Ala Pro Gly Ala His Ile Ala Ile Tyr Lys Val Cys Trp Ser Ser		
305 310 315 320		
ggc tgt tat agt tct gat ata ctt gca gca atg gat gta gct att aga	1425	
Gly Cys Tyr Ser Ser Asp Ile Leu Ala Ala Met Asp Val Ala Ile Arg		
325 330 335		
gat gga gta gac ata ttg tct ctt tca att ggt ggt ttc cct gtt cca	1473	
Asp Gly Val Asp Ile Leu Ser Leu Ser Ile Gly Gly Phe Pro Val Pro		
340 345 350		
ctt tat gag gat act att gct att ggc agt ttt cga gct atg gaa cgt	1521	
Leu Tyr Glu Asp Thr Ile Ala Ile Gly Ser Phe Arg Ala Met Glu Arg		
355 360 365		
gga att tca gtt ata tgt gct gca gga aat aat ggt cca att cta agt	1569	
Gly Ile Ser Val Ile Cys Ala Ala Gly Asn Asn Gly Pro Ile Leu Ser		
370 375 380		
tca gta gca aat gag gct cct tgg att gcc act att ggt gct agc aca	1617	
Ser Val Ala Asn Glu Ala Pro Trp Ile Ala Thr Ile Gly Ala Ser Thr		
385 390 395 400		
ctt gac agg aaa ttt cca gca ata att cag cta ggt atgtcacatt	1663	
Leu Asp Arg Lys Phe Pro Ala Ile Ile Gln Leu Gly		
405 410		
ttgtttctta aaatgatatt tgcggtgttt ccagcctaaa ttatgtgtcc ctcatccta	1723	

ttttccaaca ggt aat ggc aag tat gtg tat gga gaa tcc ttg tac ccg	1772
Asn Gly Lys Tyr Val Tyr Gly Glu Ser Leu Tyr Pro	
415 420	
ggc aaa caa gtc cat aat tct cag aaa gtt ctt gag att gtt tat ctc	1820
Gly Lys Gln Val His Asn Ser Gln Lys Val Leu Glu Ile Val Tyr Leu	
425 430 435 440	
aat gac ggt gat aat gga agt gaa ttt tgc tta aga ggg tct ctg cca	1868
Asn Asp Gly Asp Asn Gly Ser Glu Phe Cys Leu Arg Gly Ser Leu Pro	
445 450 455	
aga gct aaa gtc cat gga aaa atc gtt gta tgt gat cgt gga gtt aat	1916
Arg Ala Lys Val His Gly Lys Ile Val Val Cys Asp Arg Gly Val Asn	
460 465 470	
gga aga gca gag aaa ggt caa gtt gtt aaa gaa tca ggt ggt gtt gcc	1964
Gly Arg Ala Glu Lys Gly Gln Val Val Lys Glu Ser Gly Gly Val Ala	
475 480 485	
atg atc cta gca aat aca gca gta aat atg gag gaa gat tct gtg gac	2012
Met Ile Leu Ala Asn Thr Ala Val Asn Met Glu Glu Asp Ser Val Asp	
490 495 500	
gta cat gtc cta cct gca aca ttg att ggt ttt gac gaa tca att cag	2060
Val His Val Leu Pro Ala Thr Leu Ile Gly Phe Asp Glu Ser Ile Gln	
505 510 515 520	
ttg caa agc tat atg aac tca acg cga aaa cca aca gct cga atc ata	2108
Leu Gln Ser Tyr Met Asn Ser Thr Arg Lys Pro Thr Ala Arg Ile Ile	
525 530 535	
ttt gga gga aca gtt ata gga aaa tct agt gca cct gct gta gca caa	2156
Phe Gly Gly Thr Val Ile Gly Lys Ser Ser Ala Pro Ala Val Ala Gln	
540 545 550	
ttt tct tca agg ggt cca agt ttt act gat cct tca att ctc aaa cct	2204
Phe Ser Ser Arg Gly Pro Ser Phe Thr Asp Pro Ser Ile Leu Lys Pro	
555 560 565	
gat gtg att gct cca ggt cagtttttat tgaaccacaca attatttatt	2252
Asp Val Ile Ala Pro Gly	
570	
tagatcatag atagcaatgt gaccaacagg ttagggattc gagccgtgga atctatcatt	2312
gatgcttgaa tcagggtaga ctgcttacat cacacccttc accagaccat gcatgaaaac	2372
gggatgctct ttttatatgc atgtgaaaaa aactttaata aatagtgtaa tgttatgttt	2432
tgaacctata tottctgata actcagaatc ttgaatccgc ctctgctcca ggt gtc	2498
Val	
575	
aac ata att gct gct tgg ccg caa aat cta ggt cct agt ggc ctg gct	2536
Asn Ile Ile Ala Ala Trp Pro Gln Asn Leu Gly Pro Ser Gly Leu Ala	
580 585 590	
gag gat tca aga aga gta aac ttc act gtc tta tca gga act tca atg	2584
Glu Asp Ser Arg Arg Val Asn Phe Thr Val Leu Ser Gly Thr Ser Met	
595 600 605	
gct tgt cct cat gtt agt ggc att gct gca cta ctc cat tca att cat	2632

Ala Cys Pro His Val Ser Gly Ile Ala Ala Leu Leu His Ser Ile His	
610 615 620	
cct aaa tgg tca cca gct gca atc aaa tcc gcg cta atg aca act gca	2680
Pro Lys Trp Ser Pro Ala Ala Ile Lys Ser Ala Leu Met Thr Thr Ala	
625 630 635	
gac aca aca aac cac caa gga aaa cca atc atg gat ggt gac aca cga	2728
Asp Thr Thr Asn His Gln Gly Lys Pro Ile Met Asp Gly Asp Thr Arg	
640 645 650 655	
gct gga ctt ttc gcc ata gga gct gga cat gta aat cct gga aga tcc	2776
Ala Gly Leu Phe Ala Ile Gly Ala Gly His Val Asn Pro Gly Arg Ser	
660 665 670	
gat gat ccc gga ttg ata tat gac att aat gca aat gac tat atc act	2824
Asp Asp Pro Gly Leu Ile Tyr Asp Ile Asn Ala Asn Asp Tyr Ile Thr	
675 680 685	
cac ctt tgc act att ggt tac aaa aac tct gaa atc ctc agc att act	2872
His Leu Cys Thr Ile Gly Tyr Lys Asn Ser Glu Ile Leu Ser Ile Thr	
690 695 700	
cac aag aat gtt agc tgc cac gac gtt tta cag aaa aac agg ggt ttt	2920
His Lys Asn Val Ser Cys His Asp Val Leu Gln Lys Asn Arg Gly Phe	
705 710 715	
agt ctc aat tac ccc tct att tcc gta atc ttt aag gca gga aaa acg	2968
Ser Leu Asn Tyr Pro Ser Ile Ser Val Ile Phe Lys Ala Gly Lys Thr	
720 725 730 735	
aga aaa atg atc aca agg aga gtg aca aat gtg ggg agt cct aat tca	3016
Arg Lys Met Ile Thr Arg Arg Val Thr Asn Val Gly Ser Pro Asn Ser	
740 745 750	
atc tac tca gtt gaa att gtg gca cca gaa gga gtt aaa gtg aga gtt	3064
Ile Tyr Ser Val Glu Ile Val Ala Pro Glu Gly Val Lys Val Arg Val	
755 760 765	
aaa ccg cga cgt ctg gta ttt aaa cat gtt aat caa agt tta agt tac	3112
Lys Pro Arg Arg Leu Val Phe Lys His Val Asn Gln Ser Leu Ser Tyr	
770 775 780	
aga gtt tgg ttt ata tca agg aag aga att ggg act caa agg aga agc	3160
Arg Val Trp Phe Ile Ser Arg Lys Arg Ile Gly Thr Gln Arg Arg Ser	
785 790 795	
ttt gca gaa gga caa ttg atg tgg atc aac tcc aga gat aaa tac cag	3208
Phe Ala Glu Gly Gln Leu Met Trp Ile Asn Ser Arg Asp Lys Tyr Gln	
800 805 810 815	
aaa gtt aga agt cct att tca gtt gca tgg gca tca aag aag tga	3253
Lys Val Arg Ser Pro Ile Ser Val Ala Trp Ala Ser Lys Lys	
820 825 830	
aggctgtgcc tattttcacg tacacggaaa ataggcaggt tacgtgctat aacatatact	3313
actgatagtg taaaaattct ttatactatc agcgtagaca gtacgtaagt ngaatctatt	3373
gtcgtagcac caaagtttca ttgttcgaa catttaatta ggattctatt actatactgt	3433
atcagcacca aaagtttcat cgtcttagtt ccaaaaaattt gattaagcct ttaagttgca	3493

ttgtcttagt tccaaatcgt ttactataac ttgctgataa aaacgtaaac aattatcaaa 3553  
 tagggatttg aacattcaaa gaatttagga taaccaataa aacaataaaa aaatgaaaac 3613  
 acaaaattgt tctatttag taattttaca aatgtcaatt gttttgagaa ctcacgatt 3673  
 ttgcaataaa tttatagaag aacttgtcta ttttagtaaa atatagatca gaatttcctt 3733  
 gcttggtcaa gtttcattct tttcatttta tttcatttta aaaaaagacc caaagtttgc 3793  
 ctcataaaag aattttctct tatcatataa gagggagtta gtgctgactt tccctttatc 3853  
 atatccgtcg ac 3865

<210> 8

<211> 829

<212> PRT

<213> Solanum tuberosum

<400> 8

Ile Leu Phe Asn Pro Phe Lys Tyr Pro His Gln Ile Ile Ser Thr Asn  
 1 5 10 15

Ile Pro Leu Phe Asn Phe Lys Tyr Asn Ser Met Glu Leu Asn Phe Gln  
 20 25 30

Phe Tyr Phe Leu Cys Phe Leu Leu Cys Phe Ile Pro Leu Leu Gln Ala  
 35 40 45

Gln Asn Leu Gln Thr Tyr Ile Val Gln Leu His Pro Gln His Ala Ser  
 50 55 60

Thr Arg Thr Pro Phe Ser Ser Lys Phe Gln Trp His Leu Ser Phe Leu  
 65 70 75 80

Glu Asn Phe Thr Asn Ile Pro Leu Phe Asn Phe Lys Tyr Ile Gln Trp  
 85 90 95

Asn Ser Ile Pro Ile Leu Phe Leu Cys Phe Tyr Ser Val Tyr Ser Pro  
 100 105 110

Ala Thr Ser Ile Ser Ser Gly Glu Asn Ser Ser Ser Arg Leu Leu Tyr  
 115 120 125

Ser Tyr His Ser Ala Phe Glu Gly Phe Ala Ala Leu Leu Ser Glu Asn  
 130 135 140

Glu Leu Lys Ala Leu Lys Lys Ser Asn Asn Val Leu Ser Ile Tyr Pro  
 145 150 155 160

Glu Arg Lys Leu Glu Val Gln Thr Thr Tyr Ser Tyr Lys Phe Leu Gly  
 165 170 175

Leu Ser Pro Thr Lys Glu Gly Thr Trp Leu Lys Ser Gly Phe Gly Arg  
 180 185 190

Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Ile Trp Pro Glu Ser Pro  
 195 200 205

Ser Phe Val Asp His Gly Met Ser Pro Ile Pro Lys Lys Trp Lys Gly  
 210 215 220

Xaa Cys Gln Glu Gly Lys Asn Phe Asn Ser Ser Ser Cys Asn Arg Lys  
 225 230 235 240  
 Leu Ile Gly Ala Arg Phe Phe Gln Ile Gly His Met Met Ala Ser Lys  
 245 250 255  
 Thr Ser Lys Ser Ile Asp Phe Met Glu Asp Tyr Val Ser Pro Arg Asp  
 260 265 270  
 Ser Gln Gly His Gly Thr His Thr Ala Ser Thr Ala Gly Gly Ala Pro  
 275 280 285  
 Val Pro Met Ala Ser Val Leu Gly Asn Gly Ala Gly Glu Ala Arg Gly  
 290 295 300  
 Met Ala Pro Gly Ala His Ile Ala Ile Tyr Lys Val Cys Trp Ser Ser  
 305 310 315 320  
 Gly Cys Tyr Ser Ser Asp Ile Leu Ala Ala Met Asp Val Ala Ile Arg  
 325 330 335  
 Asp Gly Val Asp Ile Leu Ser Leu Ser Ile Gly Gly Phe Pro Val Pro  
 340 345 350  
 Leu Tyr Glu Asp Thr Ile Ala Ile Gly Ser Phe Arg Ala Met Glu Arg  
 355 360 365  
 Gly Ile Ser Val Ile Cys Ala Ala Gly Asn Asn Gly Pro Ile Leu Ser  
 370 375 380  
 Ser Val Ala Asn Glu Ala Pro Trp Ile Ala Thr Ile Gly Ala Ser Thr  
 385 390 395 400  
 Leu Asp Arg Lys Phe Pro Ala Ile Ile Gln Leu Gly Asn Gly Lys Tyr  
 405 410 415  
 Val Tyr Gly Glu Ser Leu Tyr Pro Gly Lys Gln Val His Asn Ser Gln  
 420 425 430  
 Lys Val Leu Glu Ile Val Tyr Leu Asn Asp Gly Asp Asn Gly Ser Glu  
 435 440 445  
 Phe Cys Leu Arg Gly Ser Leu Pro Arg Ala Lys Val His Gly Lys Ile  
 450 455 460  
 Val Val Cys Asp Arg Gly Val Asn Gly Arg Ala Glu Lys Gly Gln Val  
 465 470 475 480  
 Val Lys Glu Ser Gly Gly Val Ala Met Ile Leu Ala Asn Thr Ala Val  
 485 490 495  
 Asn Met Glu Glu Asp Ser Val Asp Val His Val Leu Pro Ala Thr Leu  
 500 505 510  
 Ile Gly Phe Asp Glu Ser Ile Gln Leu Gln Ser Tyr Met Asn Ser Thr  
 515 520 525  
 Arg Lys Pro Thr Ala Arg Ile Ile Phe Gly Gly Thr Val Ile Gly Lys  
 530 535 540  
 Ser Ser Ala Pro Ala Val Ala Gln Phe Ser Ser Arg Gly Pro Ser Phe  
 545 550 555 560



Thr Asp Pro Ser Ile Leu Lys Pro Asp Val Ile Ala Pro Gly Val Asn  
565 570 575

Ile Ile Ala Ala Trp Pro Gln Asn Leu Gly Pro Ser Gly Leu Ala Glu  
580 585 590

Asp Ser Arg Arg Val Asn Phe Thr Val Leu Ser Gly Thr Ser Met Ala  
595 600 605

Cys Pro His Val Ser Gly Ile Ala Ala Leu Leu His Ser Ile His Pro  
610 615 620

Lys Trp Ser Pro Ala Ala Ile Lys Ser Ala Leu Met Thr Thr Ala Asp  
625 630 635 640

Thr Thr Asn His Gln Gly Lys Pro Ile Met Asp Gly Asp Thr Arg Ala  
645 650 655

Gly Leu Phe Ala Ile Gly Ala Gly His Val Asn Pro Gly Arg Ser Asp  
660 665 670

Asp Pro Gly Leu Ile Tyr Asp Ile Asn Ala Asn Asp Tyr Ile Thr His  
675 680 685

Leu Cys Thr Ile Gly Tyr Lys Asn Ser Glu Ile Leu Ser Ile Thr His  
690 695 700

Lys Asn Val Ser Cys His Asp Val Leu Gln Lys Asn Arg Gly Phe Ser  
705 710 715 720

Leu Asn Tyr Pro Ser Ile Ser Val Ile Phe Lys Ala Gly Lys Thr Arg  
725 730 735

Lys Met Ile Thr Arg Arg Val Thr Asn Val Gly Ser Pro Asn Ser Ile  
740 745 750

Tyr Ser Val Glu Ile Val Ala Pro Glu Gly Val Lys Val Arg Val Lys  
755 760 765

Pro Arg Arg Leu Val Phe Lys His Val Asn Gln Ser Leu Ser Tyr Arg  
770 775 780

Val Trp Phe Ile Ser Arg Lys Arg Ile Gly Thr Gln Arg Arg Ser Phe  
785 790 795 800

Ala Glu Gly Gln Leu Met Trp Ile Asn Ser Arg Asp Lys Tyr Gln Lys  
805 810 815

Val Arg Ser Pro Ile Ser Val Ala Trp Ala Ser Lys Lys  
820 825

<210> 9

<211> 2492

<212> DNA

<213> Solanum tuberosum

<220>

<221> CDS

<222> (3)..(2489)

<400> 9

ga att ctg ttc aac ccc ttt aaa tac ccc cat caa att ata tca aca	47
Ile Leu Phe Asn Pro Phe Lys Tyr Pro His Gln Ile Ile Ser Thr	
1 5 10 15	
aac att cca tta ttc aac ttc aaa tat aat tca atg gaa ctg aat ttc	95
Asn Ile Pro Leu Phe Asn Phe Lys Tyr Asn Ser Met Glu Leu Asn Phe	
20 25 30	
caa ttc tat ttt ctg tgc ttt cta ctg tgt ttt att ccc ctg cta caa	143
Gln Phe Tyr Phe Leu Cys Phe Leu Leu Cys Phe Ile Pro Leu Leu Gln	
35 40 45	
gct caa aat ttg caa act tat ata gta caa tta cat cca caa cat gca	191
Ala Gln Asn Leu Gln Thr Tyr Ile Val Gln Leu His Pro Gln His Ala	
50 55 60	
tca aca aga acc cct ttt agt tct aaa ttt cag tgg cac ctt tca ttt	239
Ser Thr Arg Thr Pro Phe Ser Ser Lys Phe Gln Trp His Leu Ser Phe	
65 70 75	
ctt gaa aat ttc aca aac att cca tta ttc aac ttc aaa tat att caa	287
Leu Glu Asn Phe Thr Asn Ile Pro Leu Phe Asn Phe Lys Tyr Ile Gln	
80 85 90 95	
tgg aac tca att cca att cta ttt ctg tgt ttc tac tct gtt tat tcc	335
Trp Asn Ser Ile Pro Ile Leu Phe Leu Cys Phe Tyr Ser Val Tyr Ser	
100 105 110	
cct gct aca agc att tcc tca ggt gaa aac tgc agt tct cgc ctt ttg	383
Pro Ala Thr Ser Ile Ser Ser Gly Glu Asn Ser Ser Ser Arg Leu Leu	
115 120 125	
tac tct tac cat tct gca ttt gaa ggt ttt gca gca ctt cta tct gaa	431
Tyr Ser Tyr His Ser Ala Phe Glu Gly Phe Ala Ala Leu Leu Ser Glu	
130 135 140	
aat gag cta aag gca ctg aag aaa tgc aat aat gtg tta tca ata tat	479
Asn Glu Leu Lys Ala Leu Lys Lys Ser Asn Asn Val Leu Ser Ile Tyr	
145 150 155	
ccg gag agg aag ctt gag gtt caa aca act tat tct tac aag ttc tta	527
Pro Glu Arg Lys Leu Glu Val Gln Thr Thr Tyr Ser Tyr Lys Phe Leu	
160 165 170 175	
gga ctt agt cct aca aag gaa ggt act tgg tta aag tct gga ttt ggt	575
Gly Leu Ser Pro Thr Lys Glu Gly Thr Trp Leu Lys Ser Gly Phe Gly	
180 185 190	
cga ggc gcg atc att gga gtt ctt gat act gga att tgg cca gaa agt	623
Arg Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Ile Trp Pro Glu Ser	
195 200 205	
cca agt ttt gtt gat cat gga atg tct cct att cca aag aaa tgg aaa	671
Pro Ser Phe Val Asp His Gly Met Ser Pro Ile Pro Lys Lys Trp Lys	
210 215 220	
ggt ntc tgc caa gaa gga aaa aac ttc aat tct tca agt tgc aat cgc	719
Gly Xaa Cys Gln Glu Gly Lys Asn Phe Asn Ser Ser Ser Cys Asn Arg	
225 230 235	
aag ctt att ggt gca agg ttt ttc cag ata gga cac atg atg gca tca	767
Lys Leu Ile Gly Ala Arg Phe Phe Gln Ile Gly His Met Met Ala Ser	
240 245 250 255	

aag	aca	tca	aaa	tca	ata	gat	ttt	atg	gag	gat	tat	gta	tca	cct	cga	815
Lys	Thr	Ser	Lys	Ser	Ile	Asp	Phe	Met	Glu	Asp	Tyr	Val	Ser	Pro	Arg	
			260					265					270			
gat	tct	caa	ggc	cat	ggg	aca	cat	aca	gca	tct	act	gca	ggg	gga	gct	863
Asp	Ser	Gln	Gly	His	Gly	Thr	His	Thr	Ala	Ser	Thr	Ala	Gly	Gly	Ala	
		275					280					285				
ccc	gtt	cca	atg	gcg	agt	gtg	ctt	gga	aat	gga	gca	gga	gag	gct	cga	911
Pro	Val	Pro	Met	Ala	Ser	Val	Leu	Gly	Asn	Gly	Ala	Gly	Glu	Ala	Arg	
		290					295				300					
ggg	atg	gcc	cct	ggg	gct	cat	atc	gcg	ata	tac	aaa	gtt	tgt	tgg	tct	959
Gly	Met	Ala	Pro	Gly	Ala	His	Ile	Ala	Ile	Tyr	Lys	Val	Cys	Trp	Ser	
	305					310					315					
agt	ggg	tgt	tat	agt	tct	gat	ata	ctt	gca	gca	atg	gat	gta	gct	att	1007
Ser	Gly	Cys	Tyr	Ser	Ser	Asp	Ile	Leu	Ala	Ala	Met	Asp	Val	Ala	Ile	
	320				325				330					335		
aga	gat	gga	gta	gac	ata	ttg	tct	ctt	tca	att	ggg	ggg	ttc	cct	gtt	1055
Arg	Asp	Gly	Val	Asp	Ile	Leu	Ser	Leu	Ser	Ile	Gly	Gly	Phe	Pro	Val	
			340					345					350			
cca	ctt	tat	gag	gat	act	att	gct	att	ggc	agt	ttt	cga	gct	atg	gaa	1103
Pro	Leu	Tyr	Glu	Asp	Thr	Ile	Ala	Ile	Gly	Ser	Phe	Arg	Ala	Met	Glu	
			355				360					365				
cgt	gga	att	tca	gtt	ata	tgt	gct	gca	gga	aat	aat	ggg	cca	att	cta	1151
Arg	Gly	Ile	Ser	Val	Ile	Cys	Ala	Ala	Gly	Asn	Asn	Gly	Pro	Ile	Leu	
		370					375					380				
agt	tca	gta	gca	aat	gag	gct	cct	tgg	att	gcc	act	att	ggg	gct	agc	1199
Ser	Ser	Val	Ala	Asn	Glu	Ala	Pro	Trp	Ile	Ala	Thr	Ile	Gly	Ala	Ser	
	385					390				395						
aca	ctt	gac	agg	aaa	ttt	cca	gca	ata	att	cag	cta	ggg	aat	ggc	aag	1247
Thr	Leu	Asp	Arg	Lys	Phe	Pro	Ala	Ile	Ile	Gln	Leu	Gly	Asn	Gly	Lys	
	400				405				410					415		
tat	gtg	tat	gga	gaa	tcc	ttg	tac	cgg	ggc	aaa	caa	gtt	cat	aat	tct	1295
Tyr	Val	Tyr	Gly	Glu	Ser	Leu	Tyr	Pro	Gly	Lys	Gln	Val	His	Asn	Ser	
			420					425				430				
cag	aaa	gtt	ctt	gag	att	gtt	tat	ctc	aat	gac	ggg	gat	aat	gga	agt	1343
Gln	Lys	Val	Leu	Glu	Ile	Val	Tyr	Leu	Asn	Asp	Gly	Asp	Asn	Gly	Ser	
			435					440				445				
gaa	ttt	tgc	tta	aga	ggg	tct	ctg	cca	aga	gct	aaa	gtc	cat	gga	aaa	1391
Glu	Phe	Cys	Leu	Arg	Gly	Ser	Leu	Pro	Arg	Ala	Lys	Val	His	Gly	Lys	
		450					455					460				
atc	gtt	gta	tgt	gat	cgt	gga	gtt	aat	gga	aga	gca	gag	aaa	ggg	caa	1439
Ile	Val	Val	Cys	Asp	Arg	Gly	Val	Asn	Gly	Arg	Ala	Glu	Lys	Gly	Gln	
	465					470					475					
gtt	gtt	aaa	gaa	tca	ggg	ggg	gtt	gcc	atg	atc	cta	gca	aat	aca	gca	1487
Val	Val	Lys	Glu	Ser	Gly	Gly	Val	Ala	Met	Ile	Leu	Ala	Asn	Thr	Ala	
	480				485				490					495		
gta	aat	atg	gag	gaa	gat	tct	gtg	gac	gta	cat	gtc	cta	cct	gca	aca	1535
Val	Asn	Met	Glu	Glu	Asp	Ser	Val	Asp	Val	His	Val	Leu	Pro	Ala	Thr	

500																505																510																
ttg	att	ggt	ttt	gac	gaa	tca	att	cag	ttg	caa	agc	tat	atg	aac	tca	1583																																
Leu	Ile	Gly	Phe	Asp	Glu	Ser	Ile	Gln	Leu	Gln	Ser	Tyr	Met	Asn	Ser																																	
515																520																525																
acg	cga	aaa	cca	aca	gct	cga	atc	ata	ttt	gga	gga	aca	gtt	ata	gga	1631																																
Thr	Arg	Lys	Pro	Thr	Ala	Arg	Ile	Ile	Phe	Gly	Gly	Thr	Val	Ile	Gly																																	
530																535																540																
aaa	tct	agt	gca	cct	gct	gta	gca	caa	ttt	tct	tca	agg	ggt	cca	agt	1679																																
Lys	Ser	Ser	Ala	Pro	Ala	Val	Ala	Gln	Phe	Ser	Ser	Arg	Gly	Pro	Ser																																	
545																550																555																
ttt	act	gat	cct	tca	att	ctc	aaa	cct	gat	gtg	att	gct	cca	ggt	gtc	1727																																
Phe	Thr	Asp	Pro	Ser	Ile	Leu	Lys	Pro	Asp	Val	Ile	Ala	Pro	Gly	Val																																	
560																565																570																
aac	ata	att	gct	gct	tgg	ccg	caa	aat	cta	ggt	cct	agt	ggc	ctg	gct	1775																																
Asn	Ile	Ile	Ala	Ala	Trp	Pro	Gln	Asn	Leu	Gly	Pro	Ser	Gly	Leu	Ala																																	
580																585																590																
gag	gat	tca	aga	aga	gta	aac	ttc	act	gtc	tta	tca	gga	act	tca	atg	1823																																
Glu	Asp	Ser	Arg	Arg	Val	Asn	Phe	Thr	Val	Leu	Ser	Gly	Thr	Ser	Met																																	
595																600																605																
gct	tgt	cct	cat	gtt	agt	ggc	att	gct	gca	cta	ctc	cat	tca	att	cat	1871																																
Ala	Cys	Pro	His	Val	Ser	Gly	Ile	Ala	Ala	Leu	Leu	His	Ser	Ile	His																																	
610																615																620																
cct	aaa	tgg	tca	cca	gct	gca	atc	aaa	tcc	gcg	cta	atg	aca	act	gca	1919																																
Pro	Lys	Trp	Ser	Pro	Ala	Ala	Ile	Lys	Ser	Ala	Leu	Met	Thr	Thr	Ala																																	
625																630																635																
gac	aca	aca	aac	cac	caa	gga	aaa	cca	atc	atg	gat	ggt	gac	aca	cga	1967																																
Asp	Thr	Thr	Asn	His	Gln	Gly	Lys	Pro	Ile	Met	Asp	Gly	Asp	Thr	Arg																																	
640																645																650																
gct	gga	cct	ttc	gcc	ata	gga	gct	gga	cat	gta	aat	cct	gga	aga	tcc	2015																																
Ala	Gly	Leu	Phe	Ala	Ile	Gly	Ala	Gly	His	Val	Asn	Pro	Gly	Arg	Ser																																	
660																665																670																
gat	gat	ccc	gga	ttg	ata	tat	gac	att	aat	gca	aat	gac	tat	atc	act	2063																																
Asp	Asp	Pro	Gly	Leu	Ile	Tyr	Asp	Ile	Asn	Ala	Asn	Asp	Tyr	Ile	Thr																																	
675																680																685																
cac	ctt	tgc	act	att	ggt	tac	aaa	aac	tct	gaa	atc	ctc	agc	att	act	2111																																
His	Leu	Cys	Thr	Ile	Gly	Tyr	Lys	Asn	Ser	Glu	Ile	Leu	Ser	Ile	Thr																																	
690																695																700																
cac	aag	aat	gtt	agc	tgc	cac	gac	gtt	tta	cag	aaa	aac	agg	ggt	ttt	2159																																
His	Lys	Asn	Val	Ser	Cys	His	Asp	Val	Leu	Gln	Lys	Asn	Arg	Gly	Phe																																	
705																710																715																
agt	ctc	aat	tac	ccc	tct	att	tcc	gta	atc	ttt	aag	gca	gga	aaa	acg	2207																																
Ser	Leu	Asn	Tyr	Pro	Ser	Ile	Ser	Val	Ile	Phe	Lys	Ala	Gly	Lys	Thr																																	
720																725																730																
aga	aaa	atg	atc	aca	agg	aga	gtg	aca	aat	gtg	ggg	agt	cct	aat	tca	2255																																
Arg	Lys	Met	Ile	Thr	Arg	Arg	Val	Thr	Asn	Val	Gly	Ser	Pro	Asn	Ser																																	
740																745																750																
atc	tac	tca	gtt	gaa	att	gtg	gca	cca	gaa	gga	gtt	aaa	gtg	aga	gtt	2303																																

Ile Tyr Ser Val Glu Ile Val Ala Pro Glu Gly Val Lys Val Arg Val  
755 760 765

aaa ccg cga cgt ctg gta ttt aaa cat gtt aat caa agt tta agt tac 2351  
Lys Pro Arg Arg Leu Val Phe Lys His Val Asn Gln Ser Leu Ser Tyr  
770 775 780

aga gtt tgg ttt ata tca agg aag aga att ggg act caa agg aga agc 2399  
Arg Val Trp Phe Ile Ser Arg Lys Arg Ile Gly Thr Gln Arg Arg Ser  
785 790 795

ttt gca gaa gga caa ttg atg tgg atc aac tcc aga gat aaa tac cag 2447  
Phe Ala Glu Gly Gln Leu Met Trp Ile Asn Ser Arg Asp Lys Tyr Gln  
800 805 810 815

aaa gtt aga agt cct att tca gtt gca tgg gca tca aag aag tga 2492  
Lys Val Arg Ser Pro Ile Ser Val Ala Trp Ala Ser Lys Lys  
820 825

<210> 10

<211> 829

<212> PRT

<213> Solanum tuberosum

<400> 10

Ile Leu Phe Asn Pro Phe Lys Tyr Pro His Gln Ile Ile Ser Thr Asn  
1 5 10 15

Ile Pro Leu Phe Asn Phe Lys Tyr Asn Ser Met Glu Leu Asn Phe Gln  
20 25 30

Phe Tyr Phe Leu Cys Phe Leu Leu Cys Phe Ile Pro Leu Leu Gln Ala  
35 40 45

Gln Asn Leu Gln Thr Tyr Ile Val Gln Leu His Pro Gln His Ala Ser  
50 55 60

Thr Arg Thr Pro Phe Ser Ser Lys Phe Gln Trp His Leu Ser Phe Leu  
65 70 75 80

Glu Asn Phe Thr Asn Ile Pro Leu Phe Asn Phe Lys Tyr Ile Gln Trp  
85 90 95

Asn Ser Ile Pro Ile Leu Phe Leu Cys Phe Tyr Ser Val Tyr Ser Pro  
100 105 110

Ala Thr Ser Ile Ser Ser Gly Glu Asn Ser Ser Ser Arg Leu Leu Tyr  
115 120 125

Ser Tyr His Ser Ala Phe Glu Gly Phe Ala Ala Leu Leu Ser Glu Asn  
130 135 140

Glu Leu Lys Ala Leu Lys Lys Ser Asn Asn Val Leu Ser Ile Tyr Pro  
145 150 155 160

Glu Arg Lys Leu Glu Val Gln Thr Thr Tyr Ser Tyr Lys Phe Leu Gly  
165 170 175

Leu Ser Pro Thr Lys Glu Gly Thr Trp Leu Lys Ser Gly Phe Gly Arg  
180 185 190

Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Ile Trp Pro Glu Ser Pro

195							200				205					
Ser	Phe	Val	Asp	His	Gly	Met	Ser	Pro	Ile	Pro	Lys	Lys	Trp	Lys	Gly	
	210					215					220					
Xaa	Cys	Gln	Glu	Gly	Lys	Asn	Phe	Asn	Ser	Ser	Ser	Cys	Asn	Arg	Lys	
225					230					235					240	
Leu	Ile	Gly	Ala	Arg	Phe	Phe	Gln	Ile	Gly	His	Met	Met	Ala	Ser	Lys	
				245					250					255		
Thr	Ser	Lys	Ser	Ile	Asp	Phe	Met	Glu	Asp	Tyr	Val	Ser	Pro	Arg	Asp	
			260					265					270			
Ser	Gln	Gly	His	Gly	Thr	His	Thr	Ala	Ser	Thr	Ala	Gly	Gly	Ala	Pro	
		275					280					285				
Val	Pro	Met	Ala	Ser	Val	Leu	Gly	Asn	Gly	Ala	Gly	Glu	Ala	Arg	Gly	
	290					295					300					
Met	Ala	Pro	Gly	Ala	His	Ile	Ala	Ile	Tyr	Lys	Val	Cys	Trp	Ser	Ser	
305					310					315					320	
Gly	Cys	Tyr	Ser	Ser	Asp	Ile	Leu	Ala	Ala	Met	Asp	Val	Ala	Ile	Arg	
				325					330					335		
Asp	Gly	Val	Asp	Ile	Leu	Ser	Leu	Ser	Ile	Gly	Gly	Phe	Pro	Val	Pro	
			340					345					350			
Leu	Tyr	Glu	Asp	Thr	Ile	Ala	Ile	Gly	Ser	Phe	Arg	Ala	Met	Glu	Arg	
		355					360					365				
Gly	Ile	Ser	Val	Ile	Cys	Ala	Ala	Gly	Asn	Asn	Gly	Pro	Ile	Leu	Ser	
	370					375					380					
Ser	Val	Ala	Asn	Glu	Ala	Pro	Trp	Ile	Ala	Thr	Ile	Gly	Ala	Ser	Thr	
385					390					395					400	
Leu	Asp	Arg	Lys	Phe	Pro	Ala	Ile	Ile	Gln	Leu	Gly	Asn	Gly	Lys	Tyr	
				405					410					415		
Val	Tyr	Gly	Glu	Ser	Leu	Tyr	Pro	Gly	Lys	Gln	Val	His	Asn	Ser	Gln	
			420					425					430			
Lys	Val	Leu	Glu	Ile	Val	Tyr	Leu	Asn	Asp	Gly	Asp	Asn	Gly	Ser	Glu	
		435					440					445				
Phe	Cys	Leu	Arg	Gly	Ser	Leu	Pro	Arg	Ala	Lys	Val	His	Gly	Lys	Ile	
	450					455					460					
Val	Val	Cys	Asp	Arg	Gly	Val	Asn	Gly	Arg	Ala	Glu	Lys	Gly	Gln	Val	
465					470					475					480	
Val	Lys	Glu	Ser	Gly	Gly	Val	Ala	Met	Ile	Leu	Ala	Asn	Thr	Ala	Val	
				485					490					495		
Asn	Met	Glu	Glu	Asp	Ser	Val	Asp	Val	His	Val	Leu	Pro	Ala	Thr	Leu	
			500					505					510			
Ile	Gly	Phe	Asp	Glu	Ser	Ile	Gln	Leu	Gln	Ser	Tyr	Met	Asn	Ser	Thr	
		515					520					525				
Arg	Lys	Pro	Thr	Ala	Arg	Ile	Ile	Phe	Gly	Gly	Thr	Val	Ile	Gly	Lys	

530		535		540
Ser 545	Ser Ala Pro Ala Val Ala Gln Phe Ser 550	Ser 555	Arg Gly Pro Ser Phe 560	
Thr 565	Asp Pro Ser Ile Leu Lys Pro Asp Val 570	Ile Ala Pro Gly Val Asn 575		
Ile 580	Ile Ala Ala Trp Pro Gln Asn Leu Gly 585	Pro Ser Gly Leu Ala Glu 590		
Asp 595	Ser Arg Arg Val Asn Phe Thr Val Leu Ser Gly 600	Thr Ser Met Ala 605		
Cys 610	Pro His Val Ser Gly Ile Ala Ala Leu Leu His 615	Ser Ile His Pro 620		
Lys 625	Trp Ser Pro Ala Ala Ile Lys Ser Ala Leu Met Thr Thr Ala Asp 630	635	640	
Thr 645	Thr Asn His Gln Gly Lys Pro Ile Met Asp Gly Asp Thr Arg Ala 650	655		
Gly 660	Leu Phe Ala Ile Gly Ala Gly His Val Asn Pro Gly Arg Ser Asp 665	670		
Asp 675	Pro Gly Leu Ile Tyr Asp Ile Asn Ala Asn Asp Tyr Ile Thr His 680	685		
Leu 690	Cys Thr Ile Gly Tyr Lys Asn Ser Glu Ile Leu Ser Ile Thr His 695	700		
Lys 705	Asn Val Ser Cys His Asp Val Leu Gln Lys Asn Arg Gly Phe Ser 710	715	720	
Leu 725	Asn Tyr Pro Ser Ile Ser Val Ile Phe Lys Ala Gly Lys Thr Arg 730	735		
Lys 740	Met Ile Thr Arg Arg Val Thr Asn Val Gly Ser Pro Asn Ser Ile 745	750		
Tyr 755	Ser Val Glu Ile Val Ala Pro Glu Gly Val Lys Val Arg Val Lys 760	765		
Pro 770	Arg Arg Leu Val Phe Lys His Val Asn Gln Ser Leu Ser Tyr Arg 775	780		
Val 785	Trp Phe Ile Ser Arg Lys Arg Ile Gly Thr Gln Arg Arg Ser Phe 790	795	800	
Ala 805	Glu Gly Gln Leu Met Trp Ile Asn Ser Arg Asp Lys Tyr Gln Lys 810	815		
Val 820	Arg Ser Pro Ile Ser Val Ala Trp Ala Ser Lys Lys 825			

&lt;210&gt; 11

&lt;211&gt; 3140

&lt;212&gt; DNA

&lt;213&gt; Solanum tuberosum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(2298)

&lt;400&gt; 11

act cat tta ttc tcc ttt cta tgt ccc tta cta tgt ttt gtt tgc ata	48
Thr His Leu Phe Ser Phe Leu Cys Leu Leu Leu Cys Phe Val Cys Ile	
1 5 10 15	
caa gct caa gat ttg caa act tac ata gtt cag tta cat cca cat gga	96
Gln Ala Gln Asp Leu Gln Thr Tyr Ile Val Gln Leu His Pro His Gly	
20 25 30	
gca aca aga ccc cct ttt agc tct aaa cta caa tgg cac ctt tct ttc	144
Ala Thr Arg Pro Pro Phe Ser Ser Lys Leu Gln Trp His Leu Ser Phe	
35 40 45	
ctt gca aaa gca gct tcc tct gga gaa caa gac tcg tct tct cgt ctt	192
Leu Ala Lys Ala Val Ser Ser Gly Glu Gln Asp Ser Ser Ser Arg Leu	
50 55 60	
ttg tac tct tac cat tct gcg atg gaa ggt ttt gca gct cga ctc act	240
Leu Tyr Ser Tyr His Ser Ala Met Glu Gly Phe Ala Ala Arg Leu Thr	
65 70 75 80	
gaa gat gag gtt gag ttg tta agg gaa tct aat gat gtg ttg tcg ata	288
Glu Asp Glu Val Glu Leu Leu Arg Glu Ser Asn Asp Val Leu Ser Ile	
85 90 95	
cgt gct gag agg agg ctt gaa att cag act act tat tct tac aag ttc	336
Arg Ala Glu Arg Arg Leu Glu Ile Gln Thr Thr Tyr Ser Tyr Lys Phe	
100 105 110	
ttg gga tta agt cca acg aga gaa gga gct tgg ttg aag tct gga ttc	384
Leu Gly Leu Ser Pro Thr Arg Glu Gly Ala Trp Leu Lys Ser Gly Phe	
115 120 125	
ggt cga ggg gcg atc att gga gtg ttg gat act gga gtt tgg cca gaa	432
Gly Arg Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Val Trp Pro Glu	
130 135 140	
agt cca agt ttt gat gat cat ggg atg cca cct gct cca cag aag cgg	480
Ser Pro Ser Phe Asp Asp His Gly Met Pro Pro Ala Pro Gln Lys Trp	
145 150 155 160	
agg ggt gtc tgc caa gga gga cag gat ttt aat tct tct agt tgt aat	528
Arg Gly Val Cys Gln Gly Gly Gln Asp Phe Asn Ser Ser Ser Cys Asn	
165 170 175	
cgc aag ctt att ggt gca agg ttt ttc aga aaa gga cat cgt gtg gct	576
Arg Lys Leu Ile Gly Ala Arg Phe Phe Arg Lys Gly His Arg Val Ala	
180 185 190	
tca atg aca tca tca cca gat gca gtg gag gaa tat gtg tcg cca cgg	624
Ser Met Thr Ser Ser Pro Asp Ala Val Glu Glu Tyr Val Ser Pro Arg	
195 200 205	
gat tcc cat ggc cat ggt aca cat aca gca tcc act gct gga gga gct	672
Asp Ser His Gly His Gly Thr His Thr Ala Ser Thr Ala Gly Gly Ala	
210 215 220	
gca gtt cca ttg gct ggt gtg ctc gga aat gga gca ggg gag gct cga	720
Ala Val Pro Leu Ala Gly Val Leu Gly Asn Gly Ala Gly Glu Ala Arg	



225	230	235	240	
ggg atg gcc ccg ggt gcc cac att gca ata tat aaa gta tgc tgg ttc				768
Gly Met Ala Pro Gly Ala His Ile Ala Ile Tyr Lys Val Cys Trp Phe				
	245	250	255	
agt ggt tgt tac agc tct gat ata ctt gca gca atg gat gtg gcc atc				816
Ser Gly Cys Tyr Ser Ser Asp Ile Leu Ala Ala Met Asp Val Ala Ile				
	260	265	270	
aga gat gga gta gac ata ttg tca ctc tca ctt ggt ggc ttc cct att				864
Arg Asp Gly Val Asp Ile Leu Ser Leu Ser Leu Gly Gly Phe Pro Ile				
	275	280	285	
cca ctt tat gat gat act att gcc att gga agt ttc cga gcc atg gag				912
Pro Leu Tyr Asp Asp Thr Ile Ala Ile Gly Ser Phe Arg Ala Met Glu				
	290	295	300	
cat gga att tca gtt ata tgt gct gca ggg aat aat gga cca atc caa				960
His Gly Ile Ser Val Ile Cys Ala Ala Gly Asn Asn Gly Pro Ile Gln				
	305	310	315	320
agt tca gta gcc aac ggt gct cct tgg att gcc act att ggt gct agc				1008
Ser Ser Val Ala Asn Gly Ala Pro Trp Ile Ala Thr Ile Gly Ala Ser				
	325	330	335	
aca ctt gac agg aga ttt cca gcg tca gtt cag tta ggc aac gga aag				1056
Thr Leu Asp Arg Arg Phe Pro Ala Ser Val Gln Leu Gly Asn Gly Lys				
	340	345	350	
ttc ctg tac gga gaa tcc ttg tac cct ggg aag aaa gtt cct agc tct				1104
Phe Leu Tyr Gly Glu Ser Leu Tyr Pro Gly Lys Lys Val Pro Ser Ser				
	355	360	365	
cag aag aat ctt gag atc gtt tat gta aag gat aag gac aag gga agt				1152
Gln Lys Asn Leu Glu Ile Val Tyr Val Lys Asp Lys Asp Lys Gly Ser				
	370	375	380	
gaa ttt tgc ttg aga gga tcg cta tca aaa gca caa gtc cga ggg aaa				1200
Glu Phe Cys Leu Arg Gly Ser Leu Ser Lys Ala Gln Val Arg Gly Lys				
	385	390	395	400
atg gtt gtg tgt gat agg gga gtc aat gga agg gca gaa aaa ggc cag				1248
Met Val Val Cys Asp Arg Gly Val Asn Gly Arg Ala Glu Lys Gly Gln				
	405	410	415	
gtt gtg aag gag gca ggt ggt gct gcc atg atc tta gca aat aca gca				1296
Val Val Lys Glu Ala Gly Gly Ala Ala Met Ile Leu Ala Asn Thr Ala				
	420	425	430	
ata aat atg gag gaa gat tcc att gat gtc cat gtc ctc cca gca acg				1344
Ile Asn Met Glu Glu Asp Ser Ile Asp Val His Val Leu Pro Ala Thr				
	435	440	445	
ttg att ggc ttc gat gaa tca att caa tta caa aac tac ctg aac tca				1392
Leu Ile Gly Phe Asp Glu Ser Ile Gln Leu Gln Asn Tyr Leu Asn Ser				
	450	455	460	
aca aaa aga cca aca gct cga ttc ata ttt gga gga acg gta ata gga				1440
Thr Lys Arg Pro Thr Ala Arg Phe Ile Phe Gly Gly Thr Val Ile Gly				
	465	470	475	480
aag tct aga gca cct gca gta gct cag ttt tcg tca agg ggg cca agc				1488

Lys	Ser	Arg	Ala	Pro	Ala	Val	Ala	Gln	Phe	Ser	Ser	Arg	Gly	Pro	Ser		
				485					490					495			
tat	act	gat	ccc	tca	att	ctc	aaa	cct	gat	ttg	att	gct	cca	ggg	gta	1536	
Tyr	Thr	Asp	Pro	Ser	Ile	Leu	Lys	Pro	Asp	Leu	Ile	Ala	Pro	Gly	Val		
			500				505					510					
aac	ata	att	gcc	gct	tgg	cca	caa	aac	tta	ggc	ccc	agt	ggt	ctt	ccc	1584	
Asn	Ile	Ile	Ala	Ala	Trp	Pro	Gln	Asn	Leu	Gly	Pro	Ser	Gly	Leu	Pro		
			515				520					525					
gaa	gat	tca	cga	aga	gta	aat	ttc	act	gtc	atg	tca	ggg	acc	tca	atg	1632	
Glu	Asp	Ser	Arg	Arg	Val	Asn	Phe	Thr	Val	Met	Ser	Gly	Thr	Ser	Met		
			530			535					540						
gca	tgt	cct	cat	gta	agt	gga	att	gcc	gca	ttg	ctc	cat	tca	gct	cat	1680	
Ala	Cys	Pro	His	Val	Ser	Gly	Ile	Ala	Ala	Leu	Leu	His	Ser	Ala	His		
			545			550				555					560		
cct	aaa	tgg	act	cca	gca	gca	ata	aga	tcc	gca	tta	atg	acc	act	gca	1728	
Pro	Lys	Trp	Thr	Pro	Ala	Ala	Ile	Arg	Ser	Ala	Leu	Met	Thr	Thr	Ala		
				565				570					575				
gat	aca	gct	gat	cat	atg	gga	aaa	cca	atc	atg	gat	gga	gat	gca	cca	1776	
Asp	Thr	Ala	Asp	His	Met	Gly	Lys	Pro	Ile	Met	Asp	Gly	Asp	Ala	Pro		
			580				585						590				
gct	aaa	ctt	ttt	gca	gct	gga	gct	gga	cac	gtg	aac	cct	gga	aga	gcc	1824	
Ala	Lys	Leu	Phe	Ala	Ala	Gly	Ala	Gly	His	Val	Asn	Pro	Gly	Arg	Ala		
			595			600						605					
atc	gat	cct	gga	ttg	ata	tat	gac	atc	cag	gtt	gat	gaa	tat	atc	act	1872	
Ile	Asp	Pro	Gly	Leu	Ile	Tyr	Asp	Ile	Gln	Val	Asp	Glu	Tyr	Ile	Thr		
			610			615					620						
cat	ctt	tgc	act	atc	gga	tac	aga	aat	tct	gaa	gtc	ttc	agc	att	act	1920	
His	Leu	Cys	Thr	Ile	Gly	Tyr	Arg	Asn	Ser	Glu	Val	Phe	Ser	Ile	Thr		
				625		630				635					640		
cat	agg	aat	gtc	agc	tgc	cat	gac	att	tta	cag	aac	aac	agg	ggt	ttc	1968	
His	Arg	Asn	Val	Ser	Cys	His	Asp	Ile	Leu	Gln	Asn	Asn	Arg	Gly	Phe		
				645				650					655				
agc	cta	aat	tac	ccc	tca	att	tca	ata	act	ttc	aga	gca	gga	atg	act	2016	
Ser	Leu	Asn	Tyr	Pro	Ser	Ile	Ser	Ile	Thr	Phe	Arg	Ala	Gly	Met	Thr		
			660					665					670				
aga	aag	ata	atc	aag	agg	aga	gta	aca	aat	gtg	ggg	aac	cct	aac	tct	2064	
Arg	Lys	Ile	Ile	Lys	Arg	Arg	Val	Thr	Asn	Val	Gly	Asn	Pro	Asn	Ser		
			675				680					685					
att	tac	tca	gtt	gac	att	gag	gca	cct	gag	gga	gtc	aaa	gtg	aga	gtg	2112	
Ile	Tyr	Ser	Val	Asp	Ile	Glu	Ala	Pro	Glu	Gly	Val	Lys	Val	Arg	Val		
			690			695					700						
aag	cca	cgt	cgt	ctg	ata	ttt	aaa	cat	gtg	aac	caa	agc	tta	agc	tat	2160	
Lys	Pro	Arg	Arg	Leu	Ile	Phe	Lys	His	Val	Asn	Gln	Ser	Leu	Ser	Tyr		
				705		710			715						720		
aga	gtt	tgg	ttt	ata	tca	cga	aag	awa	ata	gag	tct	aaa	agg	atg	agc	2208	
Arg	Val	Trp	Phe	Ile	Ser	Arg	Lys	Xaa	Ile	Glu	Ser	Lys	Arg	Met	Ser		
				725				730						735			

ttt gca gag ggg caa tgg aca tgg ttc aat gta gga aac aaa gcc acg 2256  
 Phe Ala Glu Gly Gln Leu Thr Trp Phe Asn Val Gly Asn Lys Ala Thr  
                   740                  745                  750

aaa gtt aaa agt cct att tcc gtc aca tgg gca tca atg aag 2298  
 Lys Val Lys Ser Pro Ile Ser Val Thr Trp Ala Ser Met Lys  
                   755                  760                  765

tgatcactat caccactatc acaagcacca tatatttcat tgtcttagtt caaaatttcc 2358

aattaggaat ttcacatcac attataaatt gatgttagag cagatacact ttatctttcc 2418

acaaagaaga aatgatcgat aatcattgaa atgatttggtg ttttactaag tagatgtgtc 2478

tccacaatgt taagaagtat taatatgtat aaatagatta gacaaagcac gagattgccc 2538

ctgagtggag nattttctca agtttacacc ttttgaacta aattactcat aaaccagtat 2598

gacagacaaa aaattcaaga aattggcgag gcaaaagaaa acatacaata taatctcaac 2658

ttttaacaaa ttgcaagcca tttgaattag cataccgctc cataaatctc atgaacctgt 2718

cccagttctg tggagtcggc ataataact tagcttcaat tctgtcaggc tttccattaa 2778

caaacttagc attgacatca accgacgtta gaaccccttc ttcgtcaatc acgtagaatc 2838

cagtgatatc cctacttca ccagatgaat caaatacgga ggggtgatca aacctgaata 2898

tagccatacc atttgtccat cccttgactt tgtaatttc acatctggta ttgtttgtc 2958

atcagttcct tgtatgaact gaatctttgg ttgaaccatc attatacata gtctggacat 3018

ttttctggtt ttgatattgg tactgaaacg cgaacgggat aggcacaatc gttggccaat 3078

tgaatgaaga acctgcactt tgatgaacta tcttgatgc tattcctaca gtacatgaca 3138

ca 3140

<210> 12

<211> 766

<212> PRT

<213> Solanum tuberosum

<400> 12

Thr His Leu Phe Ser Phe Leu Cys Leu Leu Leu Cys Phe Val Cys Ile  
   1                  5                  10                  15

Gln Ala Gln Asp Leu Gln Thr Tyr Ile Val Gln Leu His Pro His Gly  
                   20                  25                  30

Ala Thr Arg Pro Pro Phe Ser Ser Lys Leu Gln Trp His Leu Ser Phe  
                   35                  40                  45

Leu Ala Lys Ala Val Ser Ser Gly Glu Gln Asp Ser Ser Ser Arg Leu  
                   50                  55                  60

Leu Tyr Ser Tyr His Ser Ala Met Glu Gly Phe Ala Ala Arg Leu Thr  
                   65                  70                  75                  80

Glu Asp Glu Val Glu Leu Leu Arg Glu Ser Asn Asp Val Leu Ser Ile  
                   85                  90                  95

Arg Ala Glu Arg Arg Leu Glu Ile Gln Thr Thr Tyr Ser Tyr Lys Phe  
 100 105 110  
 Leu Gly Leu Ser Pro Thr Arg Glu Gly Ala Trp Leu Lys Ser Gly Phe  
 115 120 125  
 Gly Arg Gly Ala Ile Ile Gly Val Leu Asp Thr Gly Val Trp Pro Glu  
 130 135 140  
 Ser Pro Ser Phe Asp Asp His Gly Met Pro Pro Ala Pro Gln Lys Trp  
 145 150 155 160  
 Arg Gly Val Cys Gln Gly Gly Gln Asp Phe Asn Ser Ser Ser Cys Asn  
 165 170 175  
 Arg Lys Leu Ile Gly Ala Arg Phe Phe Arg Lys Gly His Arg Val Ala  
 180 185 190  
 Ser Met Thr Ser Ser Pro Asp Ala Val Glu Glu Tyr Val Ser Pro Arg  
 195 200 205  
 Asp Ser His Gly His Gly Thr His Thr Ala Ser Thr Ala Gly Gly Ala  
 210 215 220  
 Ala Val Pro Leu Ala Gly Val Leu Gly Asn Gly Ala Gly Glu Ala Arg  
 225 230 235 240  
 Gly Met Ala Pro Gly Ala His Ile Ala Ile Tyr Lys Val Cys Trp Phe  
 245 250 255  
 Ser Gly Cys Tyr Ser Ser Asp Ile Leu Ala Ala Met Asp Val Ala Ile  
 260 265 270  
 Arg Asp Gly Val Asp Ile Leu Ser Leu Ser Leu Gly Gly Phe Pro Ile  
 275 280 285  
 Pro Leu Tyr Asp Asp Thr Ile Ala Ile Gly Ser Phe Arg Ala Met Glu  
 290 295 300  
 His Gly Ile Ser Val Ile Cys Ala Ala Gly Asn Asn Gly Pro Ile Gln  
 305 310 315 320  
 Ser Ser Val Ala Asn Gly Ala Pro Trp Ile Ala Thr Ile Gly Ala Ser  
 325 330 335  
 Thr Leu Asp Arg Arg Phe Pro Ala Ser Val Gln Leu Gly Asn Gly Lys  
 340 345 350  
 Phe Leu Tyr Gly Glu Ser Leu Tyr Pro Gly Lys Lys Val Pro Ser Ser  
 355 360 365  
 Gln Lys Asn Leu Glu Ile Val Tyr Val Lys Asp Lys Asp Lys Gly Ser  
 370 375 380  
 Glu Phe Cys Leu Arg Gly Ser Leu Ser Lys Ala Gln Val Arg Gly Lys  
 385 390 395 400  
 Met Val Val Cys Asp Arg Gly Val Asn Gly Arg Ala Glu Lys Gly Gln  
 405 410 415  
 Val Val Lys Glu Ala Gly Gly Ala Ala Met Ile Leu Ala Asn Thr Ala  
 420 425 430

Ile Asn Met Glu Glu Asp Ser Ile Asp Val His Val Leu Pro Ala Thr  
435 440 445

Leu Ile Gly Phe Asp Glu Ser Ile Gln Leu Gln Asn Tyr Leu Asn Ser  
450 455 460

Thr Lys Arg Pro Thr Ala Arg Phe Ile Phe Gly Gly Thr Val Ile Gly  
465 470 475 480

Lys Ser Arg Ala Pro Ala Val Ala Gln Phe Ser Ser Arg Gly Pro Ser  
485 490 495

Tyr Thr Asp Pro Ser Ile Leu Lys Pro Asp Leu Ile Ala Pro Gly Val  
500 505 510

Asn Ile Ile Ala Ala Trp Pro Gln Asn Leu Gly Pro Ser Gly Leu Pro  
515 520 525

Glu Asp Ser Arg Arg Val Asn Phe Thr Val Met Ser Gly Thr Ser Met  
530 535 540

Ala Cys Pro His Val Ser Gly Ile Ala Ala Leu Leu His Ser Ala His  
545 550 555 560

Pro Lys Trp Thr Pro Ala Ala Ile Arg Ser Ala Leu Met Thr Thr Ala  
565 570 575

Asp Thr Ala Asp His Met Gly Lys Pro Ile Met Asp Gly Asp Ala Pro  
580 585 590

Ala Lys Leu Phe Ala Ala Gly Ala Gly His Val Asn Pro Gly Arg Ala  
595 600 605

Ile Asp Pro Gly Leu Ile Tyr Asp Ile Gln Val Asp Glu Tyr Ile Thr  
610 615 620

His Leu Cys Thr Ile Gly Tyr Arg Asn Ser Glu Val Phe Ser Ile Thr  
625 630 635 640

His Arg Asn Val Ser Cys His Asp Ile Leu Gln Asn Asn Arg Gly Phe  
645 650 655

Ser Leu Asn Tyr Pro Ser Ile Ser Ile Thr Phe Arg Ala Gly Met Thr  
660 665 670

Arg Lys Ile Ile Lys Arg Arg Val Thr Asn Val Gly Asn Pro Asn Ser  
675 680 685

Ile Tyr Ser Val Asp Ile Glu Ala Pro Glu Gly Val Lys Val Arg Val  
690 695 700

Lys Pro Arg Arg Leu Ile Phe Lys His Val Asn Gln Ser Leu Ser Tyr  
705 710 715 720

Arg Val Trp Phe Ile Ser Arg Lys Xaa Ile Glu Ser Lys Arg Met Ser  
725 730 735

Phe Ala Glu Gly Gln Leu Thr Trp Phe Asn Val Gly Asn Lys Ala Thr  
740 745 750

Lys Val Lys Ser Pro Ile Ser Val Thr Trp Ala Ser Met Lys  
755 760 765

<210> 13  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 13  
 Gln Thr Tyr Ile Val  
 1 5

<210> 14  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 14  
 Ile Val Gln Leu His  
 1 5

<210> 15  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 15  
 Ser Ser Arg Leu Leu  
 1 5

<210> 16  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 16  
 Gln Thr Thr Tyr Ser  
 1 5

<210> 17

<211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 17  
 Ser Ser Ser Cys Asn  
 1 5

<210> 19  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 18  
 Val Leu Gly Asn Gly  
 1 5

<210> 19  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 19  
 Gly Ala His Ile Ala  
 1 5

<210> 20  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 20  
 Phe Arg Ala Met Glu  
 1 5

<210> 21  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 21

Val Ile Cys Ala Ala

1 5

<210> 22

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 22

Ala Ala Gly Asn Asn

1 5

<210> 23

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 23

Ser Ser Val Ala Asn

1 5

<210> 24

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 24

Tyr Gly Glu Ser Leu

1 5

<210> 25

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial



sequence

<400> 25

Gly Ser Glu Phe Cys  
1 5

<210> 26

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 26

Cys Leu Arg Gly Ser  
1 5

<210> 27

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 27

Arg Gly Val Asn Gly  
1 5

<210> 28

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 28

Pro Ala Thr Leu Ile Gly  
1 5

<210> 29

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 29

Ile Phe Gly Gly Thr  
1 5

<210> 30

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 30

Pro Gln Asn Leu Gly  
1 5

<210> 31

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 31

Val Asn Phe Thr Val  
1 5

<210> 32

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 32

His Val Ser Gly Ile  
1 5

<210> 33

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 33

Gly Phe Ser Leu Asn  
1 5

<210> 34  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 34  
 Arg Arg Val Thr Asn  
 1 5

<210> 35  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 35  
 Pro Asn Ser Ile Tyr  
 1 5

<210> 36  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 36  
 Leu Ser Tyr Arg Val  
 1 5

<210> 37  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 37  
 Ser Pro Ile Ser Val  
 1 5

<210> 38

<211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 38  
 Val Ile Cys Ala Ala  
 1 5

<210> 39  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 39  
 Cys Ala Ala Gly Asn  
 1 5

<210> 40  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 40  
 Ala Ala Gly Asn Asn  
 1 5

<210> 41  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 41  
 Val Ile Cys Ala Ala Gly Asn Asn Gly  
 1 5

<210> 42  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 42

Ile Ile Gly Val Leu

1 5

<210> 43

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 43

Gly Val Leu Asp Thr

1 5

<210> 44

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 44

Thr His Thr Ala Ser Thr

1 5

<210> 45

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial  
sequence

<400> 45

Ser Arg Asp Ser

1

<210> 46

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial

## sequence

&lt;400&gt; 46

Arg Asp Ser Gly

1

&lt;210&gt; 47

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: artificial  
sequence

&lt;400&gt; 47

His Val Ser Gly Ile

1

5

&lt;210&gt; 48

&lt;211&gt; 6

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: artificial  
sequence

&lt;400&gt; 48

Phe Thr Val Ser Gly Thr

1

5

&lt;210&gt; 49

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: artificial  
sequence

&lt;400&gt; 49

Ser Tyr His Ser Ala

1

5

&lt;210&gt; 50

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: artificial  
sequence

&lt;400&gt; 50

Gly Leu Ser Pro Thr  
1 5

<210> 51

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial sequence

<400> 51

Trp Leu Lys Ser Gly  
1 5

<210> 52

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial sequence

<400> 52

Phe Asn Ser Ser Ser  
1 5

<210> 53

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial sequence

<400> 53

Ala Ser Thr Ala Gly  
1 5

<210> 54

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial sequence

<400> 54

Ala Ala Met Asp Val  
1 5

<210> 55  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 55  
 Trp Ile Ala Thr Ile  
 1 5

<210> 56  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 56  
 Gly Pro Ser Gly Leu  
 1 5

<210> 57  
 <211> 6  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 57  
 Ile Ala Ala Leu Leu His  
 1 5

<210> 58  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: artificial  
 sequence

<400> 58  
 Lys Pro Ile Met Asp  
 1 5

<210> 59



<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: artificial  
sequence

<400> 59  
Val Ser Cys His Asp  
1 5

<210> 60  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: artificial  
sequence

<400> 60  
Tyr Pro Ser Ile Ser  
1 5

<210> 61  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: artificial  
sequence

<400> 61  
Ser Leu Ser Tyr Arg  
1 5